National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

ASPECTS OF BARLEY POST-ANTHESIS NITROGEN PHYSIOLOGY

by

Kayhan Foroutan-pour

August 1994

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

Department of Plant Science

Macdonald Campus of

McGill University

Montreal, Quebec

Canada

ISBN 0-612-05551-5

Description of Thesis Format

The format of this thesis is consistent with conditions approved by the Faculty of Graduate Studies and Research of McGill University and as outlined in part B, section 2 "Manuscripts and Authorship", which are as follows:

" *2/Manuscripts and Authorship: Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provding that these copies are bound as an integral part of the thesis. If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory. The thesis must still conform to all other requirements of the "Guideline Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published. The thesis must include, as separate chapters or sections: (1) a table of contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and/or summary. Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail (eg. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis. In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Oral Defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers."*

ABSTRACT

M.Sc. K. FOROUTAN-POUR PLANT SCIENCE

ASPECTS OF BARLEY POST-ANTHESIS NITROGEN PHYSIOLOGY

Barley is the world's fourth most important cereal crop, the second ranking cereal in Canada, and the most widely grown small grain cereal in Quebec. The protein concentration of cereal grains is low and the production of cereal crops with increased grain protein concentrations is desirable. This work investigates the physiological aspects of protein accumulation potential in barley grain. A recently developed perfusion system was used in four experiments conducted in 1993 and 1994. Three greenhouse experiments tested 1) four plant growth regulator (PGR): ABA at 0.01, 0.1 and 1 mg L^{-1}, kinetin at 0.1, 1 and 10 mg L^{-1}, GA_3 at 0.1, 1 and 10 mg L^{-1}, 2,4-D at 0.15, 1.5 and 15 mg L^{-1}; 2) 0, 5.4, and 10.7 mM N fertilizer solutions, applied as NH_4NO_3, and 0 and 30 mM N peduncle added N as urea; and 3) ABA at 0.01, 0.1 and 1 mg L^{-1}, kinetin at 0.1, 1 and 10 mg L^{-1}, GA_3 at 0.1, 1 and 10 mg L^{-1}, 2,4-D at 0.15, 1.5 and 15 mg L^{-1}, and 0 and 10.7 mM N fertilizer solutions, and 0 or 30 mM peduncle added N. In the field experiment, plants were allowed to take up urea at 15 or 30 mM N, or ethephon at 15 μ M. Abscisic acid and 2,4-D decreased total seed weight $spike^{-1}$. Gibberellic acid and 2,4-D increased seed protein concentration and content, while ABA decreased both of these. Kinetin and abscisic acid treatments resulted in the highest and lowest levels, respectively for flag leaf photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration. Both protein content $spike^{-1}$ and seed protein concentration were elevated in plants fertilized with 10.7 mM N via the soil and plants perfused with 30 mM N via the peduncle. Plants receiving treatments of 10.7 mM N from the soil and mixture of 30 mM N and GA_3 or 2,4-D through the peduncle had increased protein content $seed^{-1}$, and the highest seed weight $spike^{-1}$, respectively. Peduncle perfusion with 30 mM N increased spike protein concentration and content and grain protein concentration without affecting seed weight $spike^{-1}$. Grain protein concentration was increased by peduncle perfusion with ethephon. The perfusion technique worked well under field conditions.

RESUME

M.Sc. K. FOROUTAN-POUR PLANT SCIENCE

Les aspects de la physiologie de l'azote dans l'orge après la floraison

L'orge occupe la quatrième position parmi les céréales au monde, la deuxième position parmi les céréales au Canada et la première au Québec. La concentration en protéines des graines (CPG) des céréales est basse et la production de céréales à forte concentration en protéines est souhaitable. Ce travail étudie la physiologie du potentiel d'accumulation des protéines dans la graine de l'orge. Un système de perfusion développé récemment a été utilisé dans 4 expériences faites en 1993 et 1994. Trois expériences dans les serres ont étudié 1) 4 régulateurs de croissance (RC): Acide abscissique (ABA) à 0.01, 0.1 et 1 mg L^{-1}, Kinétine à 0.1, 1 et 10 mg L^{-1}, Gibbérelline (GA_3) à 0.1, 1 et 10 mg L^{-1}, 2,4-D à 0.15, 1.5 et 15 mg L^{-1}; 2) des solutions de NH_4NO_3 0, 5.4 et 10.7 mM en N ajoutées au sol, des solutions d'urée 0 et 30 mM en N ajoutées au pédoncule; et 3) ABA à 0.01, 0.1 et 1 mg L^{-1}, Kinétine à 0.1, 1 et 10 mg L^{-1}, GA_3 à 0.1, 1 et 10 mg L^{-1}, 2,4-D à 0.15, 1.5, 15 mg L^{-1}, deux solutions d'NH_4NO_3 0 et 10.7 mM en N, et deux solutions d'urée 0 et 30 mM en N. Dans le champ, les plantes ont reçu une solution d'urée 0 ou 30 mM en N, ou de l'éthephon 15 μM. L'ABA et le 2,4-D ont réduit le poids total des graines par épi (PTGE). La Gibbérelline et le 2,4-D ont élevé la concentration et la quantité absolue des protéines dans les graines, par contre ABA les a diminués. La kinétine et l'ABA ont respectivement donné les plus hautes et les plus basses valeurs pour la photosynthèse de la feuille étendard, la conductance des stomates, la transpiration et la concentration intercellulaire du CO_2. Le contenu en protéines de l'épi et la CPG ont été augmenté dans les plantes qui ont reçu 10.7 mM d'N par le sol et les plantes qui ont reçu 30 mM d'N par le pédoncule. Les plantes qui ont reçu 10.7 mM d'N par le sol et un mélange de 30 mM d'N et de GA_3 ou de 2,4-D par le pédoncule avaient respectivement une CPG élevée et le poids des graines par épis le plus élevé. La perfusion du pédoncule avec 30 mM d'N a causé une augmentation de la concentration, de la quantité absolue des protéines des épis et de la CPG sans avoir aucune influence sur le PTGE. La CPG a été élevée par une perfusion à l'éthephon. La technique de perfusion a bien fonctionné dans le champ.

ACKNOWLEDGMENTS

I appreciate the enthusiasm, curiosity and energy of my academic supervisor, Dr. Donald L. Smith, whose guidance was always available yet never interfering and willingly shared during many discussions about my research and courses. His expeditious and conscientious reviews of each version of every manuscript was constructive and very helpful. I appreciate that Dr. Katrine A. Stewart, Dr. Baoluo Ma, and Dr. Diane Mather listened carefully and provided useful suggestions at my graduate committee meetings.

I am grateful for the patience and assistance of Stewart Liebovitch, Birgit Schultz, Micheline Ayoub, Linda Nelson, and Jean-Pierre Laplaine. Appreciation is also extended to the secretaries of the Plant Science Department for their help.

I would like to thank the ministry of culture and higher education of Iran which gave me permission to attend McGill University and also provided full scholarship funds during my study.

Finally, I would like to thank my wife, Foozieh Kalhory and my daughter, Sepideh Foroutan-pour for their constant encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Section 1

LITERATURE REVIEW

1.1 Genetic Variability for Transport and Storage of Nitrogen

Obtaining good yields of high protein grain depends not only on having adequate soil nitrogen and a favourable environment but also on using a suitable genotype. Cereal cultivars have been shown to respond differently to the same levels of soil nitrogen (Barley and Naidu, 1964; Johnson et al., 1968). Such observations suggest the existence of genetic differences in the efficiency with which cereals make use of the soil nitrogen.

Peterson et al. (1975) have reported differences between wheat and oat cultivars in translocation efficiency (ratio of grain nitrogen to total plant nitrogen). Deckard et al. (1973) found significant differences in grain yield and grain protein among the different genotypes of corn. However, McNeal et al. (1966) found that the percentage of nitrogen translocated from vegetative tissue to the developing grain was constant for a wide range of wheat (Triticum aestivum L.) cultivars.

Haunold et al. (1962) suggested that an internal protein-fixing threshold exists in wheat varieties and that this threshold represents the maximum level of protein obtainable in the grain independent of yield level. However, Johnson et al. (1968) found that selected families from crosses between low and high protein types of winter wheat were more productive in both yield and protein yield than the high yielding parent.

Wild barley, Hordeum spontaneum, harbours suitable multiple sources of disease resistances, traits adaptive to environmental extremes, agronomic traits and high protein concentration. This is due to its long evolution and adaptation in the Fertile Crescent to a wide range of ecological niches (Nevo et al., 1985). In general, H. spontaneum was characterized by higher vegetative nitrogen content, and greater allocation of dry matter resources to leaves rather than stems, than to H. vulgare (Corke et al., 1989).

The grain protein to plant nitrogen relationships of barley are important because of the low nutritional value of barley protein (Doll, 1981). The hordein fraction of barley protein, which is low in lysine, usually comprises about 40% of the total grain protein at maturity, and consists mainly of two groups of polypeptides, termed hordein-1 and hordein-2 (Shewry et al., 1980). Hordein-1 increases rapidly relative to hordein-2 as nitrogen supply to the grain is increased (Giese et al., 1983). Hence the rate and duration of hordein accumulation relative to total protein, and of hordein-1 relative to hordein-2 will affect the final amount of protein in the grain, and its nutritional quality (Corke et al., 1990).

Corke et al. (1988) used a spike culture technique to ascertain whether there were basic differences between H. vulgare L. cv 'Ruth' and H. spontaneum Koch line 297. They concluded that there were no fundamental limitations in the capacity of 'Ruth' to accumulate protein while 297 appears to have a greater basal level of nitrogen availability under normal conditions.

Plant nitrogen concentration has shown positive correlations with biological yield, grain yield and grain protein yield, but there were no correlations with grain protein concentration, harvest index and nitrogen harvest index (Desai et al., 1978).

Since grain yield is proportional to a) light interception, b) efficiency of light utilization, and c) partitioning of dry matter to the grain, yield potential improvements must involve one or more of these three factors (Deckerd et al., 1985). Bulman et al. (1993d) studied genetic improvement of twenty six spring barley cultivars grown in eastern Canada from 1910 to 1988. They found that grain protein concentration decreased constantly with time and this reduction was related to an increase in the amount of nonstructural carbohydrate per grain, not to decrease in grain protein content. Since no trends were observed for N retranslocation and retranslocation efficiency, they concluded that N partitioning to the grain was not altered.

Mather et al. (1983b) applied the Griffing and the Hayman diallel methods to percent protein, protein per grain, and lysine content of grain protein in a 6x6 diallel cross of triticale (x triticale Wittmack). Their analysis showed that a) the three traits have mostly additive gene action, although some dominance was detected for percent protein and protein per grain, b) dominance for protein per grain was in the direction

of higher protein c) variation in lysine content was largely attributable to variation in percent protein, protein per grain, and grain plumpness.

1.2 Nitrogen Resources During the Grain-filling Stage

There are two possible sources of grain nitrogen in cereals: soil nitrogen usually nitrate taken up during grain filling, and redistribution of nitrogen accumulated during the vegetative stage of development (Dalling et al., 1975). In environments where the post-anthesis supply of soil N is low, and this includes most of the rain-fed crop production areas of Australia, USA, Canada, and Argentina, N redistributed from vegetative organs can contribute more than 80% of the grain N yield (Dalling, 1985). In Australia, where soil nitrogen is often unavailable to the plant at the time of grain-filling the utilization of nitrogen already in the plant is considered to be of great importance in determining the final protein content of the grain (Dalling et al., 1975). For rice, protein N in the grain is derived mainly from the N already present in the vegetative tissues at flowering (Ishizuka et al., 1953). Among the vegetative tissues of the rice plant the leaf blades have highest N content.

Leaf development and senescence are known to be under hormonal control (Ishizuka et al., 1953). Studies have indicated that peak protease activity occurs earlier, before the booting stage, in contrast to rate of leaf protein synthesis which did not begin to decrease until after booting (International Rice Research Institute, 1972). Hence, the slowing of protein synthesis coincided with the decrease in leaf N content.

With the onset of senescence, leaves change their function from being a source of mainly carbohydrates to one of mainly mineral nutrients. In the case of nitrogen and phosphorus, the amount retranslocated from vegetative parts of wheat plants is generally much higher than the amount newly taken up and delivered by the root (Martin, 1982).

During grain fill cereal plants are in general, mobilizing all reserves for deposition in seeds. Lower internodes were the main potential contributors of pre-anthesis assimilate to grain-filling but upper internodes were an important source of stored assimilate accumulated after anthesis. Crops that lost more mass from the stem also had greater grain yields. The stem is the major source of assimilate towards the

end of grain-filling (Bonnett et al., 1992). The assimilate that is stored prior to anthesis may be used to help overcome shortages of assimilate as a consequence of stress and the contribution of pre-anthesis assimilate to grain-filling could be as much as 76-100% (Gallagher et al., 1975; Gallagher et al., 1976; Scott et al., 1976; Bidinger et al., 1977)

Uptake of N during the grain-filling period in cereal crops can be considered to be a function of the availability of soil N at this time and the capacity of the roots to absorb and translocate N to the shoot (Dalling, 1985). Even under conditions where the post-anthesis soil N supply is high, at least 50% of the grain N yield will still be derived from the mobilization and redistribution of N in the vegetative organs (Spiertz et al., 1978). The leaves and stem are the most important sources of N, each contributing about 30% of the grain-N yield. The roots are a minor source, contributing less than 10% (Dalling, 1985). Dalling et al. (1976) and Waters et al., (1982) found that, the glumes are important for several reasons. Firstly, they contribute about 15% to the grain-N yield and secondly, they appear to act as a temporary sink for N during the very early phase of grain filling.

Compared to single N fertilizer application at seeding, split N application significantly increased barley grain protein concentration (Bulman et al., 1993b). Bulman et al. (1993a) found an inverse relationship between retranslocation of vegetative N to the grain and N application. Nitrogen supply to wheat kernels during grain filling depends on mobilization of N acquired during vegetative growth and on continued nitrate (NO_3^-) uptake and assimilation after anthesis. The contribution of each of these N sources during kernel growth is a function of leaf senescence, photosynthate partitioning, genotype, and soil NO_3^- level (Austin et al., 1977b; Gregory et al., 1981; Smith et al., 1983). Johnson et al. (1968) in their experiment on cereal breeding for better protein content found that rate of uptake of N in wheats with high grain protein percentage of is similar to that of low protein wheats but they translocate more leaf N to the developing grain.

1.3 Nitrogen Redistribution Along the Transport Pathway During the Grain-filling

Martin (1982) found girdling of the stem between the flag leaf and the ear of wheat plants did not change the amounts of nitrogen, phosphorus and magnesium retranslocated and transported to the ear but reduced calcium movement slightly. He also showed that a continuous connection by sieve elements between leaves (source) and grains (sink) in wheat is not a prerequisite for retranslocation of nitrogen, phosphorus or magnesium. Application of (^{15}N) to leaves in a short-term (24 h) experiment confirmed that in girdled culms nitrogen continues to move to the ear. Nitrogen is apparently easily released to the xylem. Hardy (1969) found that, after a grapevine leaf was fed $^{14}CO_2$, a selective transfer of [^{14}C] glutamine and malic acid to the xylem occurred.

Retranslocation occurs predominantly by direct transport from the leaves to the reproductive organs. However, nutrients such as nitrogen and potassium may circulate through the root during retranslocation by downward movement in the phloem, transfer to the xylem in the root, upward transport with the transpiration stream and transfer back to the phloem en route to the reproductive organs (Pate 1975). The transpiration stream in the xylem of wheat plants may contribute to retranslocation of certain nutrients from leaves to grains at those parts of the route where movement of solutes in phloem and xylem is in the same direction (Martin, 1982).

There are presently no direct measurements of solute concentration and composition along the phloem transport and associated pathways leading to sink tissues. Difficulties in obtaining samples along the pathway, whether from the sieve tubes or from other locations, have been a severe limitation. Unfortunately, phloem exudate usually cannot be collected from most plants, especially from crop species. Phloem exudate has been obtained from barley by Tully et al., (1979) and from wheat by Simpson et al., (1981); both studies employed the EDTA exudation technique in collecting phloem sap from excised leaves.

In combination with endosperm cavity sap collection, the collection of sieve tube exudate from broken grain pedicels and from severed aphid stylets established on

wheat plants have more recently provided a flexible means of sampling the transport pathway during grain filling in wheat (Fisher et al., 1984; Fisher et al., 1986a; Fisher et al., 1987).

Fisher et al. (1986b) examined gradients along the transport pathway from the peduncle to the endosperm cavity during grain-filling in wheat. They found that the sucrose concentration in the sieve tubes was almost tenfold that in the endosperm cavity sap, total amino acids were only threefold higher, and the potassium concentrations of the two were equal. Their observations also strongly implicate the movement of assimilates from the sieve tubes and across the crease tissues as important control points in grain-filling.

Mackown et al. (1986), by peduncle injection with ^{15}N-nitrate at anthesis measured in situ nitrate assimilation in winter wheat and concluded that the rapidly absorbed ^{15}N-nitrate was not incorporated uniformly into peduncle N, but the absorbed ^{15}N was readily transported to the spike. Although the relative capability of nonleaf components to assimilate soil-derived nitrate absorbed after anthesis is not indicated, it is clear that the spike and peduncle have a considerable in situ capacity to assimilate nitrate immediately following anthesis. Nitrogen-15-nitrate applied to the surface of spikes following anthesis was found to be incorporated into grain protein (Nair et al., 1977).

Harper et al., (1967) found the presence of nitrate reductase activity in the flag leaf blade and spike of winter wheat following anthesis indicates that absorbed nitrate is transported to both the flag leaf and spike. Simpson et al. (1983) found that spike transpiration equals 30 to 60% of the flag transpiration at anthesis and can exceed all leaves during the linear phase of grain filling. Therefore, it seems likely that xylem transported nitrate would reach the spike provided adequate soil nitrate is available and absorption continues (Mackown et al., 1986).

Bell et al. (1990) used ^{14}C to investigate the redistribution of assimilate in field-grown winter wheat. Their measurements of ^{14}C over the period from the start of stem elongation to the end of grain-filling show a decrease in stem dry mass. They also suggested that most respiration used current rather than stored assimilate. The mass of the stem has been shown to continue to increase for a time after anthesis

(Austin et al., 1977a; Borrell, et al., 1989; Kubauch et al., 1989). There is no evidence that pre-anthesis and post-anthesis storage of assimilate in the vegetative organs occurs by different pathways (Bonnett et al., 1992).

Barlow et al. (1983) used the liquid culture technique to investigate the influence of varying source concentrations of sucrose or glutamine on the rate of starch and protein synthesis in the grain. They concluded that: a)the entry of soluble nitrogen into the grain is relatively uncontrolled, so that each increment of nitrogen in the culture medium led to an increase in the concentration of total nitrogen and non-protein nitrogen in the grain, b) the grain is the strongest nitrogen sink in the ear, c) increasing nitrogen in the culture medium increased grain protein without affecting protein-free grain weight.

The glumes may also play an important role in the movement of N within the plant, especially the exchange of N from xylem to phloem. The glumes, leaves, and stems all have a high efficiency of N mobilization. In contrast, N in the roots is apparently not readily available or accessible for mobilization (Dalling, 1985).

1.4 Hormonal and Enzymatic Activities Effective in Redistribution of Nitrogen During Grain-filling

1.4.1 Enzymes of NO_3^- Assimilation

For most agronomic crops the principal N source is NO_3^- provided by the soil solution. This NO_3^- must be reduced to NO_2^- by nitrate reductase activity (NRA); the NO_2^- is then converted to ammonia by the activities of nitrite reductase (NiR) (Gupta et al., 1985). The ammonia is assimilated through the combined activities of glutamine synthetase and glutamate synthase (glutamine oxoglutarate aminotransferase, GOGAT) (Miflin et al., 1980; Beevers et al., 1969).

In most systems the level of extractable NRA is lower than that of the other enzymes required for NO_3^- assimilation and thus nitrate reductase (NR) must be considered the primary regulator for the input of the reduced N into the plant (Beevers et al., 1980). The presence of NO_3^- enhances production of NR and NiR at the transcriptional level whereas the observed stimulation by light appears to be exerted at the translational level (Gupta et al., 1985).

Nitrate reductase activity of the total leaf canopy, expressed as seasonal averages or converted into seasonal input of reduced N, showed a significant positive correlation with grain protein (kg N ha^{-1}), grain yield, total reduced N in the vegetative material (above ground), and grain and stover at maturity. The highest correlation between NRA and yields of grain and grain protein (kg N ha^{-1}) were obtained during the stages of ear initiation and development (Deckard et al., 1973).

Genetic studies indicate that the level of NR is highly heritable, and as a consequence progeny have been developed with predetermined NR levels by selecting parents with the appropriate NRA (Zieserl and Hageman 1962). Nitrate reductase was higher in three wheat varieties having "Atlas 66" germ plasm for high grain protein potential than "Triumph 64" a hard red winter wheat with a low grain protein potential (Rao et al., 1972). Duffield (1971) also found increases in NRA in high protein wheat varieties. Croy et al. (1970) found that a) nitrate content of the tissue was a major factor in controlling the level of NRA; b) increased NR activity from supplemental nitrogen treatments was associated with increases in grain protein (% or total). Five wheat cultivars which differed widely in their capacities to accumulate grain nitrogen were compared for seasonal patterns of leaf nitrate reductase activity. Differences in the average levels of NRA were observed between cultivars. Total seasonal NRA was closely related to total plant nitrogen at maturity. Grain nitrogen was only related to total seasonal NRA when allowance was made for significant differences between cultivars in nitrogen redistribution patterns (Dalling et al., 1975). There were significant positive correlations between NRA and total nitrogen accumulation by the plant (Eilich et al., 1973).

Oritant et al. (1971) and Wareing et al. (1967) reported that protein accumulation in the developing grain is also under hormonal control. In order for the plant to utilize the N in the vegetative organs these organs must senesce. Senescence in the cereals during the grain-filling period is referred to as monocarpic senescence (Dalling, 1985).

Dalling et al. (1975) have described the relationship between NRA and the accumulation of reduced nitrogen during vegetative development in several wheat cultivars. A highly significant relationship was found between the total level of leaf

NRA and the total plant nitrogen at maturity. Therefore it seems that, while the total level of NRA during vegetative growth may have determined the potential for accumulation of nitrogen in the grain, the extent of redistribution of nitrogen from other plant parts also exerted a major influence on the actual amount of grain nitrogen.

Zieserl et al. (1963), in experiment on NRA, protein content, and yield of four maize hybrids at varying plant populations have concluded that: A) NRA was causally related to the nitrogen metabolism of the plant since (a), top leaves had a higher NRA and protein content than bottom leaves (apparently due to shade effects) and (b) there was an inverse correlation between leaf NRA and nitrate content of all hybrids, B) a parallel was shown between leaf NRA, and protein content and physiological stages of development as determined by seasonal sampling.

1.4.2 Proteases

In cereals the role of proteolytic enzymes may be of special importance in the transfer of protein from the vegetative tissues to the developing grain (Frith et al., 1975). High nitrate reductase before flowering and high leaf protease activities after flowering appear to be related to high grain protein production (Rao et al., 1972). Protease activity in the leaf is also higher in wheats with high grain protein than in low protein wheats. Accumulation of grain nitrogen was studied in the wheat cultivars Argentine IX and Insignia. The pattern of nitrogen removal from several tissues of each cultivar was compared with the pattern of acid proteinase activity. There was a highly significant relationship between the rate of nitrogen loss from the tissues and the rate estimated from the enzyme activity measurements (Dalling et al., 1976).

Rao et al. (1971) found that protease levels in juvenile wheat plants were greater for a 'high' grain protein variety (NB#65317, a selection from a cross between 'Atlas66' and 'Comanche') than for a ''low'' protein variety ('Triumph 64', C.I. 12132) during early seedling growth.

Developing grains of rice cultivars with high percentages of protein tended to have higher levels of soluble protein, free amino N, and protease, and a faster rate of leucine incorporation than grain with average percentages of protein, regardless of

grain yield (Perez et al., 1973).

1.4.3 Plant Growth Regulator Effects

Several studies indicated that auxin herbicides increased seed protein and starch content (Martin et al., 1990; Bangerth et al., 1985; Rademacher et al., 1984; Hewitt et al., 1968).

Alpha-amylase is a Ca^{2+}-containing protein that is synthesized on the rough endoplasmic reticulum. Gibberellin induces the formation of the enzyme α-amylase by increasing Ca^{+2} flux into the endoplasmic reticulum in the aleurone layer of the barley grain (Bush et al., 1993; Hewitt et al., 1968).

Abscisic acid (ABA) decreases both Ca^{+2} flux into the endoplasmic reticulum and the amount of calcium that accumulates in the endoplasmic reticulum of barley aleurone cells in vivo (Bush et al., 1993).

Ma et al. (1992b) investigated post-anthesis ethephon (2-chloroethyl phosphoric acid, an ethylen source) effects on the yield of two barley cultivars, Cadette and Leger, during three years. They found that post-anthesis application of ethephon extended the grain-filling period by 1 to 3 d with variation among both cultivars and years. They concluded that under climatic conditions such as those prevalent in northeastern North America post-anthesis application of ethephon can potentially enhance grain fill and yield of spring barley (Ma et al., 1992b).

Plant growth regulator (PGR) application does not increase total dry matter accumulation on a per-shoot basis, but does affect dry matter partitioning patterns, leading to alterations in grain yield that may be positive or negative depending on the condition during a given crop year (Ma et al., 1992d). Bulman and Smith (1993c) examined the effects of ethephon and triadimefon on yield and grain protein concentration of spring barley. They concluded that ethephon can influence grain protein concentration by altering the protein and nonprotein components of the grain. Under greenhouse conditions, ethephon application increased protein accumulation in the barley grain (Ma et al., 1994a).

1.5 Techniques for Experimentation in Grain Nitrogen Accumulation and Findings

Lack of suitable methods for the study of nutritional and metabolic requirements of developing grain in cereal crops has been a major problem impeding progress in this field.

Grabau et al. (1986) used stem infusion into lower internodes of soybean plants grown throughout seed development under both greenhouse and field conditions to improve the methionine concentration of soybean (Glycine max [L.] Merr) seed storage protein. Their results showed that methionine supplementation of intact soybean plants improved protein quality through change in storage protein composition.

Stewart et al. (1953) investigated the formation of cellulose in wheat plants by using precursors glucose-1-^{14}C, sorbitol-1-^{14}C, and succinic acid-2, 3-^{14}C, which were administered to the plant aseptically by injection of sterile solutions into the hollow internodes.

McConnel et al. (1956) studied transport of carbon-14 injected into the hollow stems of growing wheat plants in the form of sodium acetate-1-^{14}C and -2-^{14}C.

Mather et al. (1984) cultured barley spikes of the cultivar Bomi and high-lysin mutants Risφ 1508 and Risφ 56 on liquid media at different levels of N and sucrose. They concluded that: a) Bomi accumulated N in response to increasing N levels in the medium and a higher level was reached than in spikes of intact plants, b) endosperm dry weight and starch were lower than in intact plants and declined at higher N levels, c) the mutants had lower dry weights and starch contents, and higher sucrose contents than Bomi, d) the distribution of N in salt-soluble, hordein, and non-protein N fractions appeared to be normal, e) uptake of culture medium by the spikes was affected by both N and sucrose concentration, f) at high N levels, the mutants accumulated less hordein, and more non-protein N than Bomi.

Soares et al. (1986) grew barley plants (cv. Clipper) to maturity in pH-controlled, aerated solution culture with 2 mM inorganic ^{14}N supplied as nitrate alone, ammonium alone or 1:1 nitrate plus ammonium. When seed filling was well advanced, the ^{14}N feeding solutions were replaced by ^{15}N feeding solutions, and the

plants were grown in these media for 6 and 12 h before harvesting. Nitrogen-15 analyses of the plant material showed that even at this late developmental stage, barley plants were still accumulating and assimilating considerable quantities of N, although at a slower rate than in younger plants. They also concluded that the poor organic ^{15}N accumulation observed in NO_3^--fed plants was due to slow absorption rate of NO_3^- ions relative to NH_4^+ ions and the low NO_3^- reducing potential of the vegetative organs of the plant at this stage of development.

Plant-induced changes in nutrient solution pH varied with the form of N in the solution. When NO_3^- was the only form of N present, pH levels consistently increased from the initial level. However, solution pH decreased when NH_4^+ was present in quantities as low 0.5% of the total N (Dodge and Hiatt, 1972).

Mackown and Sanford (1986) used a injection technique to study nitrate assimilation in winter wheat. In this technique selected culms were injected once only with 100 μM of solution.

Ma and Smith (1992c) recently reported the use of a peduncle perfusion technique to deliver nitrogen solutions continuously during grain-filling into barley. Their results show that the peduncle perfusion system is capable of delivering large quantities of substances to the developing grain of hollow-stemmed grasses. In addition, they mentioned several advantages in using internode or peduncle injection techniques to monitor nutrient metabolism, assimilate partitioning and protein synthesis: nutrients can be added at specific times and in specific concentrations, assimilation is rapid, combinations of materials (nutrients, radiolabeled effectors, plant growth regulators) can be added, and it is less damaging to the plant than leaf feeding. Nitrogen is one of the most important and easily mobile macronutrients and is therefore suitable for testing a stem perfusion technique (Ma and Smith, 1992c and 1994b).

1.6 Suitability of Barley for Nitrogen Physiology Investigations

In terms of total production, barley is the fourth most important food cereal (Rasmusson, 1985). Since most agriculture is conducted under limiting soil and climatic conditions, studies of barley resistance to abiotic stresses are important. One

of the important attributes of barley is salinity tolerance. While not a halophyte, barley tolerates high salt concentrations in the soil (Levitt 1972; Ballantyne 1962). However, it has been reported that exposure of barley plants to high salt concentrations alters some biochemical parameters of the cells (Stance et al., 1992). Pesci (1989) has observed accumulation of proline in barley leaf segments treated with ABA and KCl or NaCl. Accumulation of glycine and betaine in wheat plants subjected to salt stress has also been observed by McDonnel and Wyn Jones (1988). Because the peduncle perfusion technique (Ma and Smith 1992c) involves flooding the peduncle cavity with solutions that may have reasonably high osmotic levels tolerance to salt stress is important.

Cultivated barley, is an important experimental subject for genetic and plant breeding studies of the cereal crops. Geneticists and plant breeders have looked to barley because of genetic characters such as: 1) its diploid nature, which allows the classification and analysis of numerous hereditary characters, 2) low chromosome number (2n=14), 3) relatively large chromosomes, which permit detection of many kinds of chromosome aberrations, 4) high degree of self fertility, and 5) ease of hybridization (Rasmusson, 1985).

Because it is diploid mutants are much more easily identified than in polyploid crops such as wheat or oat. Such mutants can be invaluable in physiological studies.

1.7 Hypotheses

The general hypothesis is that barley plants have the physiological capacity to store substantially more protein in grain than is normally observed and that application of N solutions and/or some plants growth regulators to barley plants during the grain filling period will lead to substantial increases in the accumulation of protein in grain.

Specific hypotheses are: 1) the roots are a bottle-neck for N entry into cereal plants, 2) the addition of some plant growth regulators will substantially alter the distribution of N among plant parts, 3) the stimulatory effects of peduncle perfused PGR and N (urea), and soil N on seed protein concentration are additive, 4) the peduncle perfusion technique can be applied to field grown plants and will produce

essentially the same results as have already been observed in controlled environment produced plants.

1.8 Objectives

The general objective is to use the peduncle perfusion technique to investigate aspects of overall cereal crop N and related metabolisms and the physiological effects of changes in this metabolism.

The specific objectives are to investigate the effect of post-anthesis N and/or plant growth regulator addition through the peduncle coupled with various levels of N addition to the root environment on the N dynamics of:

1) grain through effects on seed number, seed weight per spike and seed, grain protein concentration and spike protein content,

2) mainstem and flag leaf through effects on weight and protein concentration at physiological maturity, and flag leaf net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration.

Preface to Section 2

Section 2 is material intended for a manuscript by K. Foroutan-pour and D.L. Smith which will be submitted for publication in the **Annuals of Botany**. The format has been changed to conform as much as possible with the guidelines set by the Faculty of Graduate Sciences and to be consistent within this thesis. Tables and Figures are presented immediately after they have been discussed. References are listed in a separate section at the end of the thesis.

In this section we address the effects of soil N, peduncle N and Plant growth regulator on accumulation of protein in grain.

Section 2

PROTEIN ACCUMULATION POTENTIAL IN BARLEY GRAIN AS AFFECTED BY SOIL AND PEDUNCLE APPLIED N, AND PEDUNCLE APPLIED PLANT GROWTH REGULATORS

2.1 ABSTRACT

In this study, the peduncle perfusion system was used to deliver a range of plant growth regulators (PGR) and/or N solutions to barley plants during the grain filling period. Three experiments were conducted to determine the physiological and developmental effects of 1) peduncle administered PGR, 2) combinations of soil and peduncle applied N, and 3) the combination of soil applied N, and peduncle administered N and PGR on barley plants during grain filling. Aspects of N and dry matter accumulation and photosynthesis were measured. The perfusion technique floods the peduncle interior with a treatment solution for period of weeks to months, allowing the plant to take up administered substances from the perfused solution. The first experiment tested four PGR: ABA at 0.01, 0.1 and 1 mg L^{-1}, kinetin at 0.1, 1 and 10 mg L^{-1}, GA_3 at 0.1, 1 and 10 mg L^{-1}, 2,4-D at 0.15, 1.5 and 15 mg L^{-1}. The second experiment tested three levels of soil N fertility (0, 5.4, and 10.7 mM N fertilizer solution applied as NH_4NO_3), and two concentrations of peduncle added N (0 and 30 m M N as urea). The third experiment tested four PGR: ABA at 1 mg L^{-1}, kinetin at 10 mg L^{-1}, GA_3 at 10 mg L^{-1}, 2,4-D at 15 mg L^{-1}, and two concentrations of soil N fertility (0 and 10.7 mM N), and two concentrations of peduncle added N (0 and 30 m M N). Distilled water controls were included in each experiment. The volumes of solution taken up ranged from 21 to 174 mL $plant^{-1}$, with an average of value of 110 mL $plant^{-1}$. For experiment 1, ABA and 2,4-D decreased total seed weight of the perfused spike. Protein concentration and content per seed of plants receiving 2,4-D and gibberellic acid were highest and those of plants receiving ABA were lowest. The frequency and degree of PGR effects were greatest for the top third

of the spike. Kinetin and abscisic acid treatments generally resulted in the highest and lowest levels, respectively, for flag leaf net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration. For experiment 2, both seed protein content spike^{-1} and seed protein concentration in plants fertilized with 10.7 mM N via the soil and plants perfused with 30 mM N via the peduncle were higher than other treatments. For experiment 3, plants receiving treatments of 10.7 mM N from the soil and mixture of 30 mM N and GA_3 or 2,4-D through the peduncle had increased protein content per seed, and the highest total seed weight produced by the perfused stem, respectively. Consequently, they both increased grain protein concentration and spike protein content.

2.2 INTRODUCTION

It has been demonstrated that protein accumulation in the developing grain is under hormonal control (Oritant et al., 1971; Wareing et al., 1967), and is influenced by the amount of N available (Ma et al., 1992c, 1994b). However, the effect of hormonal activities on accumulation of protein in seed has not been widely investigated. Among the PGRs ethephon and 2,4-D have been most studied.

The herbicide 2,4-D frequently affects the distribution of protein in plants and sometimes, as might be expected from its stimulating effect on nitrate reduction, it increases the total content of organic nitrogen (Hewitt et al., 1968). Nitrate reduction, the first stage of NO_3^- assimilation by plants, is carried out by nitrate reductase (Salsac et al., 1987). Martin et al. (1990) investigated effects of herbicides applied at three growth stages on spring wheat (Triticum aestivum L.). They found that auxin based herbicides increased seed protein content, particularly when applied at Zadoks growth stage (ZGS) 44. Proline and lysine content were not influenced by herbicide treatment at any stage of application. Indol-3-acetic acid (IAA) has been reported to increase stomatal aperture and stimulate photosynthesis (Davies et al., 1987; Guinn et al. 1993). There is evidence that IAA alters the pattern of weight per seed between and within the ear (Bangerth et al., 1985; Rademacher and Graebe 1984).

Ma et al. (1994a, 1994b) have already investigated the effects of peduncle perfused ethephon (ethylene) on barley seed protein accumulation. They found that ethephon can increase protein components in grain.

Kaufman et al. (1983) and Shewry (1992) found that the two major effects of GA in barley and several other plant species, are (i) stimulation of internode elongation in the culm or stem, with corresponding effects on leaf shape and inflorescence characteristics, and (ii) stimulation of grain germination and seedling growth. Stem and leaf extension are plant responses to increased levels of endogenous gibberellins (Kirby et al., 1970). The only investigations of gibberellins and seed protein involve α-amylase. Gibberellin induces the formation of α-amylase by increasing Ca^{+2} flux into the endoplasmic reticulum in the aleurone layer of barley seeds (Bush et al., 1993; Hewitt et al., 1968).

Davies et al. (1987) found that cytokinins increased stomatal aperture and as a result, increased photosynthesis. Michael et al. (1972) and Herzong (1982) found that there is a relationship between grain size of wheat and barley and cytokinin level, especially during the initial two weeks after anthesis. If cytokinin effects on seed size alter one of the two major components (carbohydrate and protein) more than the other, the seed protein concentration will be affected. There have been no investigations of cytokinin effects on seed protein accumulation.

Abscisic acid (ABA) is best known as a growth inhibitor (Walton 1980). It has been reported that ABA decreases stomatal aperture and, as a result, decreases photosynthesis (Seemann et al., 1987; Austin et al., 1986). Koshkin et al., (1990) found an inverse relationship between seed ABA concentration and seed size when comparing the most recent and oldest wheat cultivars. The ABA concentration per ear increased from the older to the modern cultivars. However, Bousquet et al., (1990) found no relationship between ABA content and 1000-grain weight in wheat. Abscisic acid decreases both Ca^{+2} flux into the endoplasmic reticulum and the amount of calcium that accumulates in the endoplasmic reticulum of barley aleurone cells in vivo (Bush et al., 1993). Rademacher et al. (1984) found that the maximum ABA concentration was higher in smaller-grained spring wheat cultivars. There have been no investigations of ABA effects on seed protein accumulation.

Soil nitrogen and redistribution of nitrogen in the plant are normally the two possible sources of nitrogen in cereal plants during the grain-filling period (Dalling et al., 1975). In addition, under experimental conditions peduncle perfused solutions can be a third nitrogen source (Ma et al. 1992c, 1994b).

Grain protein concentration was increased by application of N fertilizer to corn (Zhang et al., 1993; Ippersil et al., 1989), oat (Portch et al., 1968), wheat (Doyle et al., 1991; Gooding et al., 1991; Warder et al., 1963), and barley (Bulman et al., 1993b and 1993a; Birch et al., 1990; Kucey, 1987).

A technique for continuously administering PGR substances has recently been developed by Ma and Smith (1992c) for hollow-stemmed plants such as barley. Prior to development of this technique there was no method for the study of long term perturbation of cereal carbon and N metabolisms by direct addition of substances. For instance, Mater et al. (1984) used the substantially more disruptive spike culture method, but felt that the carbon metabolism of the isolated spikes was not normal. Others have attempted such studies in tissue culture (Soares et al. 1986; Dodge et al. 1972), an even more artificial circumstance. Ma and Smith (1992c) used the peduncle perfusion technique to supply N containing solutions to barley plants during the grain filling period. They found that this system is capable of delivering large quantities of substances to developing cereal grains under normal growing conditions and without trauma to the plants. Ma et al. (1992c and 1994b) found that peduncle perfused nitrogen solutions increased seed nitrogen compared with nonperfused or distilled water perfused controls. They did not investigate the combined effects of high soil N fertility and peduncle perfused N.

Since protein synthesis is under hormonal control, it can be hypothesized that a combination of plant growth regulators which stimulate barley grain protein accumulation, peduncle perfused nitrogen, and soil supplied nitrogen could lead to expression of the maximum protein accumulation potential of barley seeds.

Three experiments were conducted to determine the physiological and developmental effects of 1) PGR, 2) combinations of soil and peduncle N and 3) the combination of selected soil N levels, peduncle N levels and PGR administered during grain filling on the physiology and development of barley plants and the ability of

barley grain to accumulate protein.

2.3 MATERIALS AND METHODS

Three experiments were carried out in the greenhouse of the Plant Science Department of McGill University, Ste Anne de Bellevue, QC, Canada. The rooting medium of each experiment was 1:2:3:3 mixture (by volume) of peat, vermiculite, promix and soil. The plants were grown in pots that were 155 mm in diameter and 150 mm deep (a 2.5 L pot). Five seeds of Leger, a spring barley cultivar widely grown in eastern Canada, were planted in each pot. The seedlings were thinned to one per pot when the plants reached Zodaks growth stage (ZGS) 12, (two leaves unfolded) (Zadoks et al., 1974). The pots were examined daily and watered whenever necessary. Artificial light was used to complement natural light when the natural light was not sufficient. Each experiment was arranged in a randomized complete block design with three blocks and one replication of each treatment in each block.

A syringe perfusion system was set up when the plants reached ZGS 65 (anthesis). For each main stem, the flag leaf sheath was carefully opened to expose the peduncle for the maximum possible length without injury. Two 26-gauge needles were inserted into the peduncle at a 45° angle. The first needle was approximately 5 cm below the collar and served to allow air to escape during flooding of the peduncle. The second needle was 5 to 10 cm below the first and 2 to 10 cm above the flag leaf node. The peduncle surface and the needle were surrounded by a triangle of masking tape to form a cup against the side of the peduncle. The cup was filled with fluid latex (Vultex, General Latex Canada, QC), which dried over the course of several days, sealing the needle to the peduncle.

Solutions were added to the system when the latex was completely dry (after about 48 h). A flexible plastic tubing (Tygon i.d. 0.8 mm, o.d. 2.4 mm) was connected to the needles and fixed with silicone seal. These needles were the standard disposable type (Becton Dickinson, Rutherford, NJ), with the plastic portion removed. A 60-mL syringe barrel fitted with a 21-gauge needle was attached to the tubing leading to the lower needle. This syringe acted as a reservoir of the solution

to be tested and was held above the spike. The top of the syringe barrel was covered with a stopper to avoid loss of solution through evaporation and stop insects, etc. from falling into the reservoir. A needle was inserted through the stopper to allow air to enter into the space inside the syringe. The open end of the other tube was held above the top of the syringe barrel.

When solution was added to the syringe barrel, it flowed into the bottom of the peduncle, through the peduncle, and up the tube at the peduncle top. The level of the solution in the syringe barrel reservoir and the upper tube were always the same. The whole system was attached to a bamboo support stake with a rubber band. In each experiment, 1 or 2 of the 18 peduncles initially injected showed signs of leakage or blockage. This potential problem was dealt with by equipping more plants than were needed with the perfusion apparatus. After a solution was added to the syringe barrel, injected peduncles were examined until maturity to make sure that solution was moving out of the syringe reservoir and that there were no leaks.

In these experiments, data were collected 1) during the plant development [daily amount of absorbed solution, flag leaf photosynthesis (only in experiments 1 and 3) and time of maturity], and 2) at harvest [total amount of absorbed solution, total seed weight, grain weight, mainstem and flag leaf weight (not measured in experiment 1), concentration of protein in mainstem, flag leaf and seed (concentration of protein in mainstem and flag leaf was not measured in experiment 1), and seed protein concentration in bottom, middle and upper thirds of the spike (only in experiment 1)]. The spike was divided into three portions because previous work has shown that the distal region of spike is quite sensitive against changes induced by PGR (ethephon) application (Ma et al., 1994a).

Grains were ground with a Udy cyclone mill (Udy Corp., Fort Collins, CO) fitted with a 1 mm screen. The resulting flour samples were digested with sulphuric acid plus S-type Kjeltab catalyst [Tecator Manual, Kjeltec System 1002 Distilling Unit (Tecator, Höganäs, Sweden)] and the resulting NH_4 was distilled into H_3BO_3 and quantified by titration with dilute acid (AACC, 1983; Bradstreet, 1965). Results were expressed as average weight per grain, and grain N concentration (g kg^{-1}).

Net photosynthesis (μmol m^{-2} s^{-1}), transpiration (mol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}), and intercellular CO_2 concentration (mM) were measured by the LI-COR 6200 photosynthesis system (Li-Cor, Lincoln, Nebraska). Measurements were started ten days after injection and repeated once a week for three weeks. In the case of net photosynthesis and transpiration rate, quantum density (μmol m^{-2} s^{-1}) and leaf temperature (°C), respectively were included in the statistical models as covariable terms.

The data were analyzed with GLM and MANOVA (repeated measure design) procedures of the SAS package (SAS Institute, 1985), according to Steel and Torrie (1980). The amount of solution delivered into a peduncle was used as a covariable for the analysis of average grain weight and grain N concentration. Significance between individual means was determined by protected least significant difference ($P \leq 0.05$) analysis.

2.3.1 Experiment 1

The first experiment was planted 20 February 1993 and harvested on 26 June 1993. Two and half grams (2.5 g L^{-1}) of 20-20-20 N-P-K commercial fertilizer was added to each pot after thinning, at tillering, and at plant heading. Except at maturity the plants always appeared green and healthy. Treatments were imposed, on 13 April approximately 3 days after the mainstem heading stage.

The experimental design was 5 by 3 factorial. Four PGR, including ABA at 0.01, 0.1 and 1 mg L^{-1}, kinetin at 0.1, 1 and 10 mg L^{-1}, GA_3 at 0.1, 1, and 10 mg L^{-1}, 2,4-D at 0.15, 1.5 and 15 mg L^{-1}, were tested. These solution were added from several days after heading to harvesting. A distilled water control was also included. Since ethephon (ethylene) has already been tested with the peduncle perfusion technique (Ma et al., 1994a, 1994b), it was not included in this work. The intermediate level of each PGR is the standard one used in plant tissue culture, while the low and high levels are an order of magnitude above and below the intermediate level.

2.3.2 Experiment 2

The second experiment was planted 5 May 1993 and harvested on 11 September 1993. Treatments were imposed, on 2 August approximately 3 days after the mainstem heading stage.

The experimental design was a 3 by 2 factorial. The factors were soil N fertility [0, 5.4, and 10.7 mM N as $NH_4(NO_3)$ added, in volumes sufficient to saturate the soil, at four growth stages: three leaf, tillering, heading, and grain-filling] and concentration of peduncle added N (0 and 30 m M N as urea, perfused from the heading stage until end of the grain filling). The 10.7 mM N level was selected as this is equal to 150 mg N L^{-1} or, given a 25 % pore space (usual in most soils) and from waterings with the N fertilizer solution this equals 375 mg N $plant^{-1}$. At a seeding rate of 300 plants m^{-2} this equals 150 kg N ha^{-1}, a very high level for cultivars grown in eastern Canada (Bulman and Smith 1993b). Bulman and Smith (1993b) found that application of N fertilizer above 150 kg N ha^{-1} does not increase seed protein. The 30 mM N level was selected for peduncle perfusion as high levels were found to cause salt damage (Ma and Smith 1992c).

2.3.3 Experiment 3

The third experiment was planted 13 November 1993 and harvested on 15 Febrary 1994. Treatments were imposed, on 25 January 1994 approximately 3 days after the mainstem heading stage.

The experimental design was a 5 by 2 by 2 factorial. Four PGR, including ABA at 1 mg L^{-1}, kinetin at 10 mg L^{-1}, GA_3 at 10 mg L^{-1}, and 2,4-D at 15 mg L^{-1}, were tested. These PGR were selected as they produced the most interesting effects in experiment 1. Also included was distilled water as a control. Two concentrations of N were applied to the soil, these were 0 and 10.7 mM N as NH_4NO_3 added, in volumes sufficient to saturate the soil, at five stages: seedling growth, three leaf, tillering, heading, grain-filling. Two concentrations of peduncle applied N (0 and 30 mM N as urea) were applied from the heading stage until physiological maturity stage.

2.4 RESULTS AND DISCUSSION

2.4.1 Solution uptake

In this experiment, the peduncle perfusion system developed by Ma and Smith (1992c) was used to deliver various PGR to barley plants during the grain filling period. The average volume of peduncle solution taken up was 125.9 mL per peduncle for experiment 1, 120.1 mL per peduncle for experiment 2 and 84.3 mL per peduncle for experiment 3. The perfusion periods were 66, 40 and 20 days for experiments 1, 2 and 3, respectively. In experiment 3, the ABA solution was taken up in higher volumes and, the kinetin solution in lower volumes than distilled water. Compared to previous studies (Ma et al. 1992c , 1994b) average uptake by plants in experiments 1 and 2 were high. This large uptake volumes may have been due to the long time periods during which perfusion occurred. Plants were allowed to absorbed solution until maturity, while, Ma et al. (1992c, 1994b) kept plants on the perfusion system for 20 to 30 days.

Table 2.1 shows the means $\pm$ standard error of solutions absorbed. Variation in volume ranged from 91 to 152 mL, 94 to 138 mL and 21 to 174 mL for experiments 1, 2 and 3, respectively. For each plant, lowering the open end of the upper tube to the level of the peduncle resulted in solution dripping from that tube at a rate of several mL per minute, indicating that the variation between plants in rate of solution uptake was not due to restrictions on the rate of solution delivery to the peduncle, but rather to differences in solution absorption between plants. In these experiments the volume of solution absorbed was not a significant covariable for average weight per seed and grain protein concentration. Differences in the uptake volume between the perfused solutions are probably due to the greater osmotic potential of the more concentrated solutions.

The average daily uptake of solutions by plants was maximal at the beginning of the perfusion period with fluctuation during the first few days followed by an approximately linear decline of -0.06 mL day^{-1} in experiment 1, -0.12 mL day^{-1} in experiment 2, and -0.11 mL day^{-1} for experiment 3 (Fig 2.1). A similar decline was not noted by Ma and Smith (1992c, 1994b).

Table 2.1. Absorption of perfused solution through the peduncles of barley plants (values are mean ± s.e.).

Treatment	Experiment 1	Experiment 2	Experiment 3
Plant growth regulator	-----mL-----	-----mL-----	-----mL-----
2,4-D	124.1 ± 5.7	----	91.9 ± 8.5
Gibberellic acid (GA_3)	121.6 ± 6.6	----	80.8 ± 7.9
Kinetin	118.0 ± 5.2	----	34.3 ± 2.8
Abscisic acid (ABA)	120.8 ± 4.9	----	125.4 ± 11.7
Distilled water	145.2 ± 1.8	----	89.0 ± 7.3
Level[T]			
Low	139.1 ± 2.3	----	----
Intermediate	127.1 ± 3.7	----	----
High	111.6 ± 4.4	----	----
N Soil (NH_4NO_3)			
10.7 mM	----	120.8 ± 3.6	83.7 ± 7.5
5.4 mM	----	124.8 ± 4.1	----
Distilled water	----	114.7 ± 7.0	84.9 ± 7.4
N Peduncle (Urea)			
30 mM	----	112.2 ± 3.5	74.4 ± 6.5
Distilled water	----	128.0 ± 3.1	93.2 ± 7.9

[T] The intermediate level is the standard one used in plant tissue culture, while the low and high levels are an order of magnitude above and below the intermediate level, respectively.

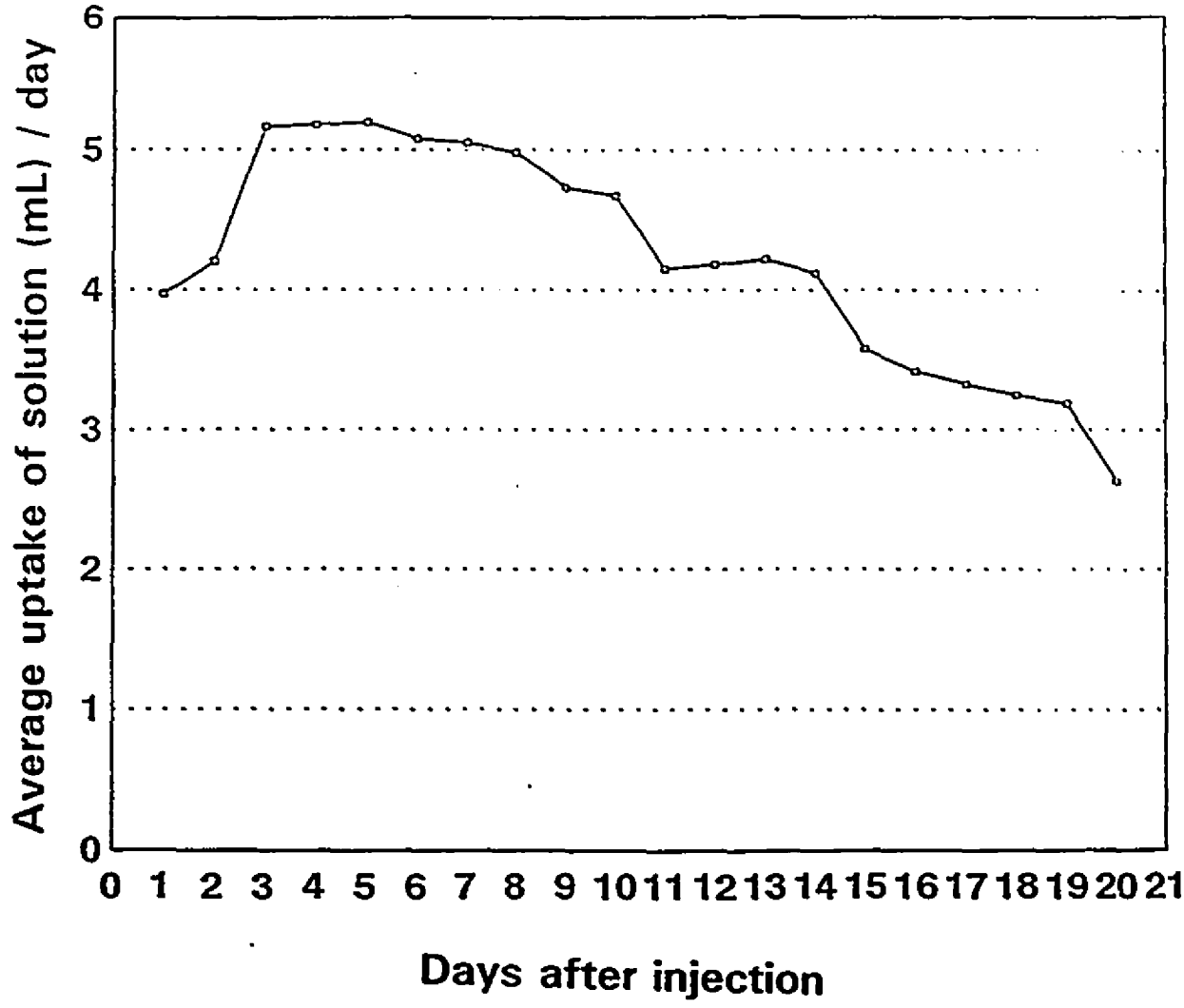

Fig. 2. 1. Average daily uptake of solution for all plants from the 1st to 20th day after injection.

2.4.2 Growth and development

2.4.2.1 Experiment 1

Number of seeds produced on the perfused stem of plants treated with 2,4-D and ABA was lower than other treatments (Table 2.2). Higher concentrations of ABA and lower concentrations of IAA were found in aborted kernels than nonaborted kernels of maize (Zea mays L.) (Reed et al., 1989). Therefore abscisic acid may promote kernel abortion after kernel formation has been initiated. There were no differences between various PGR for weight per seed (Table 2.2).

Differences were found among PGRs for total seed weight produced by the spike of the perfused stem. Abscisic acid and 2,4-D caused spike seed weight to decrease compared to other treatments. These effects are due to peduncle seed number. The IAA effects are surprising as Bangerth et al. (1985) found a positive correlation between IAA concentration and dry weight accumulation rate in grain. A similar correlation was reported by Rademacher et al. (1984) between accumulation of starch in grain and IAA grain content. In contrast, these workers found a negative correlation between grain size and ABA grain concentration.

Mean grain protein content varied among treatments (Table 2.2). Accumulation of protein in the grain of plants receiving 2,4-D and gibberellic acid through the peduncle was higher than that of plants receiving ABA. However, only the grain protein contents in plants receiving 2,4-D was different from, and in this case higher than, that of plants treated with distilled water. Martin et al. (1990) investigated 2,4-D effects on spring wheat and found that auxin herbicides promoted seed protein concentration, especially when applied at the boot stage.

Net photosynthesis (μmol m^{-2} s^{-1}), transpiration (mol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}), and intercellular CO_2 concentration (mM) were not different among the applied PGR (data not shown). Transpiration and stomatal conductance were higher at the intermediate solution concentrations than the high solution concentrations.

Table 2.2. Mean seed number, seed weight produced by the spike of the infused stem, grain protein concentration, spike protein content, weight per seed, protein content per seed, and seed number, seed weight and spike protein content produced by the middle 7 nodes of the infused stem spike for experiment 1.

Treatment	Measurement+								
	Entire Spike						Second 7 nodes		
PGR+	SN	GPC -mg g^{-1}-	SW --g--	SPC --mg--	WS --mg--	PCS --mg--	SN	SW --g--	SPC --mg--
2,4-D	14.1+ b	157.1+ a	0.49+ b	75.9+ b	34.1	5.4+ a	22.9+ b	0.80+ ab	117.5+ b
GA_3	16.4 a	156.9 a	0.56 a	87.9 a	33.9	5.3 ab	25.3 a	0.86 a	142.7 a
Kinetin	16.4 a	149.6 b	0.56 a	83.2 ab	33.7	5.1 bc	23.8 ab	0.79 ab	117.1 b
ABA	13.8 b	142.5 c	0.46 b	65.5 c	33.4	4.8 c	21.8 b	0.72 b	102.6 c
DW	16.4 a	148.3 b	0.56 a	83.0 ab	33.9	5.1 bc	23.2 b	0.77 ab	113.4 bc
F test ⊢	***	***	***	***	NS	**	*	*	***
Level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Level X PGR	NS	NS	NS	NS	NS	NS	NS	NS	NS
C.V.⊣	7.3	3.4	9.5	10.5	4.8	6.2	9.1	11.3	12.6

+ PGR = plant growth regulator; GA_3 = gibberellic acid; ABA = abscisic acid; DW = distilled water

✚ Measurement notation: SN = seed number; GPC = grain protein concentration; SW = seed weight per spike; SPC = spike protein content; WS = weight per seed; PCS = protein content per seed;

✚ Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

⊢ NS, *, **, or *** For the overall model; no significant difference or different at the 0.05, 0.01 and 0.001 level of probability, respectively.

⊣ Coefficient variation (%)

Previous research into PGR effects on stomatal aperture and photosynthesis rate generally found that ABA decreases stomatal aperture and, as a result, decreases photosynthesis (Seemann et al., 1987). In contrast, indol-3-acetic acid (IAA) and cytokinins increased stomatal opening and stimulated photosynthesis (Davies et al., 1987; Austin et al., 1986). In addition, Ma et al. (1992a) found that ethephon application to spring barley caused an increase in penultimate leaf photosynthetic rates a few days after ethephon application.

Mean seed number, seed weight and spike protein content for each section of the spike (top, middle, bottom) were analyzed separately (Tables 2.2 and 2.3). There were interaction effects between PGR types and levels for the bottom 7 and top nodes for seed number, total seed weight and total protein content. No interaction effect was observed at the middle 7 nodes of the spike.

Seed number, total seed weight and total seed protein content of the middle 7 spike nodes were lowered when plants were treated with ABA and increased when plants received GA_3 (Table 2.2). Reed et al. (1989) concluded that, after completion of flower abortion at the ear tip, aborted kernels contained higher concentrations of ABA and lower concentrations of IAA than non-aborted kernels. Thus, ABA may increase kernel abortion at the spike tip. High values for seed number, total seed weight and total protein for the lowest 7 nodes were observed for plants treated with GA_3 at the highest concentration. At the lowest concentration GA_3 treatment resulted in low seed number and seed weight in each spike section (Table 2.3). Kinetin at the intermediate concentration caused low total protein accumulation for each spike section. Low values of this variable at the top nodes were found when plants received 2,4-D at the intermediate concentration.

Data on seed number, total seed weight per spike and total spike section protein content (Tables 2.2 and 2.3) were also analyzed by repeated measure design (Crowder and Hand 1990) to test for differences between different sections of spike. There were highly significant differences between the three spike sections. There were often no differences between PGRs, at the spike bottom and middle, but at the tip of the spike differences occurred frequently.

Table 2.3. Mean seed number, seed weight per spike and spike section protein content at various combinations between PGRs and levels for first and top nodes for experiment 1.

Treatment		Bottom nodes			Top nodes		
PGR[+]	Level[¬]	Seed number	Seed weight per spike ---g---	Spike protein content --mg--	Seed number	Seed weight per spike ---g---	Spike protein content --mg--
2,4-D	High	13.0[+] d	0.44[+] de	75.2[+] bc	7.0[+] de	0.23[+] d	34.1[+] cde
	Intermediate	14.7 abcd	0.53 abcd	92.2 ab	2.7 g	0.08 f	12.1 f
	Low	14.7 abcd	0.51 abcd	92.7 ab	6.7 de	0.22 d	33.6 cde
GA_3	High	15.7 a	0.59 a	104.1 a	9.7 bc	0.31 bc	38.9 bcd
	Intermediate	15.7 a	0.56 ab	100.5 a	10.7 ab	0.35 ab	46.2 ab
	Low	13.0 d	0.43 d	73.5 bc	7.7 de	0.24 cd	30.9 de
Kinetin	High	14.0 abcd	0.48 abcd	78.2 bc	11.7 ab	0.37 ab	54.1 a
	Intermediate	13.7 bcd	0.47 bcd	70.9 c	11.3 ab	0.37 ab	52.7 a
	Low	15.3 ab	0.58 a	89.9 abc	10.7 ab	0.35 ab	51.6 a
ABA	High	14.0 abcd	0.46 bcd	71.2 c	5.3 ef	0.18 de	22.8 ef
	Intermediate	15.0 abc	0.51 abcd	73.8 bc	5.3 ef	0.17 de	23.1 ef
	Low	15.0 abc	0.55 ab	86.7 abc	4.0 fg	0.13 ef	17.1 f
DW	High	14.7 abcd	0.54 abc	86.1 abc	11.7 ab	0.37 ab	53.9 a
	Intermediate	15.0 abc	0.55 ab	91.2 abc	10.7 ab	0.35 ab	44.8 abc
	Low	13.3 de	0.46 bcd	74.6 bc	12.3 a	0.39 a	54.2 a
F test[⊢]		*	**	*	**	**	**
C.V.[⊣]		7.6	12.2	14.5	16.0	16.6	17.8

[+] PGR = plant growth regulator; GA_3 = gibberellic acid; ABA = abscisic acid; DW = distilled water

[‡] Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

[⊢] NS, *, **, or *** For the overall model; no significant difference or different at the 0.05, 0.01 and 0.001 level of probability, respectively.

[⊣] Coefficient variation (%)

[¬] The intermediate level is the standard one used in plant tissue culture, while the low and high levels are an order of magnitude above and below the intermediate level, respectively.

The rank of three spike sections for seed number, total seed weight and total protein content were middle > bottom > top, in all three cases. The ranking of mean grain weight and grain protein concentration were both bottom > middle > top. Ma et al. (1994a) found that the rank for grain weight was middle > bottom > top, and bottom > middle = top section of the spike for grain N concentration.

2.4.2.2 Experiment 2

There were no differences among treatments applied through the peduncle for seed number. However, seed number differed with soil applied N level. Number of seeds in plants fertilized by 10.7 mM N was higher than in other treatments (Table 2.4). Given that seed number is established at anthesis and that the soil N treatments were applied before anthesis, and that peduncle N treatments were applied after anthesis, this result seems reasonable.

Total seed weight produced by a perfused stem and weight per seed were generally not affected by treatments applied in the soil or treatments applied in the peduncle. Total seed weight per spike was an exception, being higher than plants treated with 10.7 mM soil applied N (Tables 2.4). The average weight per seed was 33.4 mg. No difference for weight per seed of plants perfused with various N concentrations was reported by Ma et al. (1992c, 1994b).

Plants receiving 10.7 mM soil N or 30 mM peduncle N had higher protein content and concentration per seed than other treatments. Given that the 10.7 mM N treatment is approximately equal to 150 kg N ha^{-1}, a level above which the protein concentration of barley no longer responds to increased soil N fertility (Bulman and Smith 1993b), the increased seed protein content and concentration when peduncle perfused N is added to treatment receiving 10.7 mM soil fertility suggests that the accumulation of barley seed N is limited by the ability of the plant roots, rather than the seeds, to take up N.

Both treatments imposed in the soil and peduncle affected spike protein content and grain protein concentration (Table 2.4). Plants perfused with 30 mM N through the peduncle had higher spike and grain protein concentrations than plants perfused with distilled water. Similar results were reported by Ma et al. (1992c, 1994b). Both

Table 2.4. Mean seed number, seed weight produced by the spike of the infused stem, grain protein concentration, spike protein content, weight per seed, protein content per seed, flag leaf weight, flag leaf protein concentration, mainstem weight, and mainstem protein concentration for experiment 2.

Treatment	Measurement*									
N Soil (NH_4NO_3)	SN	GPC mg g^{-1}	SW -g-	SPC -mg-	WS -mg-	PCS -mg-	FW -g-	FPC mg g^{-1}	MW -g-	MPC mg g^{-1}
10.71 mM	58.5+ a	169.3+ a	2.0+ a	327.8+ a	33.3	5.6+ a	0.26+ a	115.7+ a	1.8+ a	53.4+ a
5.36 mM	55.0 ab	115.5 b	1.9 ab	217.7 b	33.8	3.9 b	0.21 b	75.7 b	1.6 b	34.3 b
DW+	50.5 b	110.7 b	1.7 b	186.1 b	33.3	3.7 b	0.19 b	68.5 b	1.6 b	17.8 c
F test⊢	*	***	NS	***	NS	***	***	***	*	***
N Peduncle (Urea)										
30 mM	55.1	153.6+ a	1.9	285.6+ a	33.6	5.2+ a	0.23	125.2+ a	1.6	38.4+ a
DW+	54.2	110.0 b	1.8	202.2 b	33.3	3.7 b	0.22	48.0 b	1.7	31.9 b
F test⊢	NS	***	NS	***	NS	***	NS	***	NS	*
N Soil X N Peduncle	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C.V.⊣	7.9	4.5	11.3	10.2	7.1	6.4	7.4	9.2	6.1	13.2

+ DW = distilled water

* Measurement notation: SN = seed number; GPC = grain protein concentration; SW = seed weight per spike; SPC = spike protein content; WS = weight per seed; PCS = protein content per seed; FW = flag leaf weight of mainstem; FPC = flag leaf protein concentration of mainstem; MW = mainstem weight; MPC = mainstem protein concentration

+ Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

⊢ NS, *, **, or *** For the overall model; no significant difference or different at the 0.05, 0.01 and 0.001 level of probability, respectively.

⊣ Coefficient Variation (%)

total spike protein content and grain protein concentration in plants fertilized with 10.7 mM N via the soil were higher than in plants receiving 5.4 mM N or distilled water. Bulman and Smith (1993b) found that higher rates of nitrogen fertilizer applied at seeding or at awn emergence increased the amount of protein per grain, grain protein concentration, plant N concentrations, total plant N accumulation, and grain N accumulation. They also found that applying some of the N at seeding and some at heading increased grain protein concentration compared to a single application of equivalent N amounts at seeding.

There were no interactions between nitrogen levels applied in soil and nitrogen concentrations perfused into the peduncle.

There were no differences among peduncle N treatments for flag leaf weight and mainstem weight. However, the highest soil N level increased flag leaf and mainstem weight over other soil N treatments (Table 2.4).

Flag leaf and mainstem protein concentrations showed differences at various nitrogen levels in the soil and peduncle. Flag leaf and mainstem protein concentrations at 10.7 mM N in soil and 30 mM N in the peduncle were higher than at other levels (Table 2.4). Ma et al. (1994b) also reported elevated protein concentrations in vegetative tissue due to peduncle perfusion with N containing solutions.

Plants grown in soil receiving 10.7 mM N and peduncle perfused with 30 mM N had high protein levels in the grain, flag leaf and mainstem. This suggests that although the plants exhibited no nitrogen deficiency, with the addition of N through the peduncle, translocation from vegetative tissues to grain was not complete.

In this study, maximum average protein concentration in grain was 189.0 mg g^{-1}. This maximum was found in plants receiving treatments of 10.7 mM N from soil and 30 mM N through the peduncle. Some barley genotypes grown in spike culture in a growth medium containing N, showed up to 350 mg g^{-1} protein concentration per seed (Corke and Atsmon, 1988 and 1990). However, Mather et al. (1984) felt that carbohydrate deposition in seeds of spike cultured stems was not complete, which could lead to high protein concentrations. The maximum grain protein concentration found in plants perfused with 30 mM N (urea) and receiving more standard levels of

soil N fertility was 153.6 mg g^{-1} (Ma and Smith 1992c). Our highest seed protein concentration in the absence of peduncle perfused N was 169.3 mg g^{-1}, thus contributing of both soil and peduncle N sources for barley plant in this experiment improved grain protein concentration by 117%. Also, in this experiment plants receiving 10.7 mM N from soil and 30 mM N via the peduncle showed 26% increase in grain protein concentration compared to plants receiving only 10.7 mM N from soil. This suggests that although there was no interaction between nitrogen levels in the soil and the peduncle, additional vegetative growth and N storage in vegetative tissues may increase the content of N translocated to grain.

2.4.2.3 Experiment 3

In this experiment, plants receiving 10.7 mM N via the soil grew better and had a healthier appearance than plants receiving distilled water. In addition, plants fertilized with 10.7 mM N reached the heading stage later than plants receiving distilled water in the soil.

Plant growth regulators can affect photosynthetic CO_2 uptake either by affecting stomatal aperture or by affecting the activity of photosynthetic enzymes. In most cases, we found no interaction between PGR, peduncle nitrogen and soil nitrogen concentrations for flag leaf stomatal conductance, transpiration, net photosynthesis and intercellular CO_2 concentration (Table 2.5).

There were differences between PGR for flag leaf net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration. In most cases, kinetin and abscisic acid resulted in the highest and lowest rates, respectively (Table 2.5). The single exception was ABA treatment which resulted in the highest value for net photosynthesis. It has been previously reported that IAA and cytokinins promote stomatal opening (Davies and Mansfield, 1987; Incoll and Jewer, 1987). Conversely, ABA can close stomata and also decrease the activity of ribulose 1,5-bisphosphate carboxylase, resulting in decreased photosynthesis (Seemann and Sharkey 1987). Ma et al. (1992a) found that penultimate leaf photosynthesis rates increased slightly immediately after ethephon application, and there was a tendency toward increased photosynthetic rates thereafter.

Table 2.5. Mean flag leaf net photosynthesis, stomatal conductance, transpiration and intercellular concentration of barley plants as affected by interaction between PGRs, peduncle and soil nitrogen concentrations for experiment 3.

			Measurement+			
Plant growth regulator	N Peduncle (Urea)	N Soil (NH_4NO_3)	NP $\mu mol\ m^{-2}\ s^{-1}$	SC $mol\ m^{-2}\ s^{-1}$	T $mmol\ m^{-2}\ s^{-1}$	IC (CO_2) mM
2,4-D	30 mM	10.7 mM	11.2+ bcd	0.37+defgh	8.1+ defgh	8.6 + bcd
		D.W.	13.2 abc	0.59 bcde	12.5 bcd	9.4 abc
	D.W.	10.7 mM	14.1 abc	0.44 defg	9.4 defg	9.8 ab
		D.W.	6.1 e	0.32 efgh	7.4 efgh	9.7 abc
Gibberellic acid (GA_3)	30 mM	10.7 mM	13.6 abc	0.27 fgh	6.5 fgh	7.7 d
		D.W.	8.6 de	0.51 cdefg	11.4 bcde	9.5 abc
	D.W.	10.7 mM	15.2 ab	0.50 cdefg	10.0 cdefg	8.5 bcd
		D.W.	12.2 abcd	0.86 ab	14.8 ab	9.2 abc
Kinetin	30 mM	10.7 mM	15.1 ab	0.52 cdefg	9.9 defg	9.2 abc
		D.W.	11.1 bcd	0.89 a	15.4 ab	9.7 abc
	D.W.	10.7 mM	14.6 abc	0.64 abcd	11.8 bcde	8.8 abcd
		D.W.	12.6 abcd	0.91 a	18.6 a	10.3 a
Abscisic acid (ABA)	30 mM	10.7 mM	11.8 abcd	0.23 gh	6.6 fgh	7.8 d
		D.W.	14.9 ab	0.41 defg	10.0 cdefg	8.9 abcd
	D.W.	10.7 mM	15.6 a	0.24 fgh	6.3 gh	7.8 d
		D.W.	8.7 de	0.12 h	4.0 h	7.8 d
Distilled water	30 mM	10.7 mM	10.6 cd	0.52 cdef	11.0 bcdef	10.1 a
		D.W.	14.1 abc	0.79 abc	14.2 abc	9.5 abc
	D.W.	10.7 mM	12.1 abcd	0.43 defg	8.8 defg	8.3 cd
		D.W.	11.7 abcd	0.73 abc	15.3 ab	10.1 a
PGR X N Peduncle			NS	*	*	NS
PGR X N Soil			NS	NS	NS	NS
N Peduncle X N Soil			**	NS	NS	NS
PGR X N Peduncle X N Soil			**	NS	NS	NS
C.V.‡			20.3	33.8	26.2	9.6

+ Measurement notation: NP = net photosynthesis; SC = stomatal conductance; T = transpiration; IC = intercellular concentration (CO_2)

NS, or *, ** For the overall model; no significant or significant at the 0.05, and 0.01 probability levels, respectively.

+ Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

‡ Coefficient variation (%)

We found that ABA caused a decrease in flag leaf stomatal conductance, transpiration and intercellular CO_2 concentration, but did not decrease flag leaf net photosynthesis (Table 2.5). This suggests that while stomates were more closed some aspect of photosynthesis inside the flag leaf functioned more efficiently and compensated for the increased stomatal resistance.

Compared to distilled water, plants receiving 10.7 mM soil N had higher flag leaf net photosynthesis. Conversely, flag leaf stomatal conductance, transpiration and intercellular CO_2 concentration in plants fertilized with 10.7 mM N in soil were lower than plants treated with distilled water. The combination of higher photosynthetic rates, more closed stomates and lower intercellular CO_2 concentrations indicates that the cause of the higher photosynthetic rates for the 10.7 mM soil N treated plants was due to better uptake of CO_2 inside the leaves.

There was no difference between 30 mM N and distilled water peduncle perfusion for net photosynthesis, stomatal conductance, transpiration, and intercellular CO_2 concentration (Table 2.5).

We did not observe PGR effects on photosynthetic variables in experiment 1, but we did in this experiment. The level of N fertility in experiment 1 was intermediate for all treatments. All the PGR related photosynthetic effects observed in experiment 3 occurred at very high (soil N plus peduncle N) or very low (no N fertilizer solutions added) N fertility.

PGR by peduncle nitrogen concentration interactions occurred for seed protein concentration (mg g^{-1}) (Table 2.6). In all cases but one, the seed protein concentration of plants perfused with PGR combined with nitrogen solutions was significantly higher than those of plants perfused with the same PGR but combined with distilled water. The exception was the kinetin treatment for which there was no difference when combined with perfused nitrogen or distilled water.

Generally there were no interactions between PGR and nitrogen concentrations in the soil or in the peduncle for mainstem protein content. The exception was a PGR by soil nitrogen concentration interaction (Table 2.7). Mainstem protein concentrations were different among the various PGR, soil nitrogen treatments and

Table 2.6. Mean seed number produced by the spike of the infused stem, seed protein concentration, mainstem protein concentration and flag leaf protein concentration of barley plants receiving various interaction of PGRs, soil and peduncle nitrogen concentrations for experiment 3.

Plant growth regulator	N Peduncle (Urea)	N Soil (NH_4NO_3)	Seed number	Seed protein C[T] mg g^{-1}	Mainstem protein C mg g^{-1}	Flag leaf protein C mg g^{-1}
2,4-D	30 mM	10.7 mM	75.3[+] a	171.3[+] abc	60.7[+] bc	173.9[+] a
		D.W.	63.3 bcde	147.3 def	11.8 gh	88.3 g
	D.W.	10.7 mM	57.0 defg	147.7 def	52.4 de	120.7 ef
		D.W.	65.0 abcd	107.4 ij	8.5 h	20.1 i
Gibberellic acid (GA_3)	30 mM	10.7 mM	40.0 hij	178.8 a	63.1 bc	169.7 ab
		D.W.	59.3 def	154.5 cde	11.7 gh	163.4 ab
	D.W.	10.7 mM	75.3 a	136.0 fgh	60.8 bc	137.5 cde
		D.W.	60.3 def	98.0 j	10.6 h	50.5 h
Kinetin	30 mM	10.7 mM	72.3 abc	153.5 def	63.5 b	135.4 cde
		D.W.	50.0 fgh	120.8 hi	9.9 h	50.1 h
	D.W.	10.7 mM	47.3 ghi	153.9 cde	55.5 cde	128.9 e
		D.W.	29.3 j	127.9 gh	11.9 gh	43.5 h
Abscisic acid (ABA)	30 mM	10.7 mM	74.0 ab	163.0 abcd	55.9 bcde	150.7 bcd
		D.W.	50.3 fgh	177.2 a	19.1 fg	127.3 e
	D.W.	10.7 mM	38.0 ij	145.4 defg	49.3 e	105.3 fg
		D.W.	55.0 defg	108.8 ij	9.7 h	37.2 hi
Distilled water	30 mM	10.7 mM	53.0 efg	173.1 ab	74.1 a	153.9 bc
		D.W.	62.0 cde	157.4 bcd	20.9 f	124.4 ef
	D.W.	10.7 mM	55.0 defg	139.5 efg	59.9 bcd	132.9 de
		D.W.	66.0 abcd	91.3 j	9.7 h	31.9 hi
PGR X N Peduncle			***	***	NS	***
PGR X N Soil			***	NS	*	***
N Peduncle X N Soil			NS	***	NS	***
PGR X N Peduncle X N Soil			***	*	NS	***
C.V.[4]			11.8	7.6	13.4	11.3

[T] C = Concentration

NS, or *, *** For the overall model; no significant or significant at the 0.05 and 0.001 probability levels, respectively.

[+] Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

[4] Coefficient variation (%)

Table 2.7. Mean seed weight produced by the spike of the infused stem, spike protein content, weight and protein content per seed of barley plants as affected by interaction between PGRs, peduncle and soil nitrogen concentrations for experiment 3.

Plant growth regulator	N Peduncle (Urea)	N Soil (NH_4NO_3)	Seed weight per spike —g—	Protein content per seed —mg—	Weight per seed —mg—	Spike protein content —mg—
2,4-D	30 mM	10.7 mM	2.1[+] ab	4.8[+] bcd	27.8[+] efg	354.7[+] a
		D.W.	1.6 defg	3.8 ef	25.7 g	237.8 ef
	D.W.	10.7 mM	1.8 bcde	4.6 bcde	30.9 bcdef	259.1 de
		D.W.	1.7 cdef	2.8 g	26.3 fg	183.7 g
Gibberellic acid (GA_3)	30 mM	10.7 mM	1.4 ghi	6.1 a	33.9 abc	242.6 c
		D.W.	1.7 defg	4.4 cde	28.3 defg	258.5 de
	D.W.	10.7 mM	2.4 a	4.3 cde	31.8 abcde	324.8 ab
		D.W.	1.8 cde	2.8 g	28.9 defg	171.2 gh
Kinetin	30 mM	10.7 mM	2.0 bc	4.3 cde	28.1 defg	311.1 abc
		D.W.	1.6 defg	3.9 def	32.9 abcd	197.4 fg
	D.W.	10.7 mM	1.6 defg	5.3 ab	34.7 ab	251.9 c
		D.W.	1.0 i	4.6 bcde	36.0 a	131.4 h
Abscisic acid (ABA)	30 mM	10.7 mM	1.9 bcd	4.3 cde	26.3 fg	317.0 abc
		D.W.	1.4 fgh	5.0 bc	28.2 defg	244.5 c
	D.W.	10.7 mM	1.2 hi	4.6 bcde	31.7 abcde	175.1 gh
		D.W.	1.6 defg	3.2 fg	29.7 cdefg	175.9 gh
Distilled water	30 mM	10.7 mM	1.6 efg	5.3 ab	30.5 bcdefg	277.9 cde
		D.W.	1.9 bcde	4.9 bc	31.1 bcdef	302.8 bcd
	D.W.	10.7 mM	1.9 bcde	4.8 bcd	34.7 ab	263.5 de
		D.W.	1.9 bcd	2.7 g	29.8 cdefg	178.1 g
PGR X N Peduncle			***	***	NS	***
PGR X N Soil			**	***	*	*
N Peduncle X N Soil			NS	*	NS	**
PGR X N Peduncle X N Soil			***	***	NS	NS
C.V.[ǂ]			11.8	11.2	9.7	12.2

NS, or *, **, *** For the overall model; no significant or significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

[+] Within a column means followed by the same letter are not different by a protected $LSD_{0.05}$.

[ǂ] Coefficient variation (%)

peduncle applied N levels. The mainstem protein concentration for 10.7 mM soil N and 30 mM N in peduncle were higher than for PGR and distilled water.

All possible interactions between PGR and nitrogen concentration in the soil and in the peduncle occurred for flag leaf protein content. Plants treated with combinations of 2,4-D, 30 mM peduncle N, and 10.7 mM soil N, or GA_3, 30 mM peduncle N, and 10.7 mM soil N had highest flag leaf protein concentrations.

Once again higher protein concentrations in grain, flag leaves and mainstems of plants peduncle perfused with 30 mM N and receiving 10.7 mM N soil suggests that there is no nitrogen sink limitation when peduncle N is supplied. Similar results were found in experiment 2 and by Ma et al. (1994b). Nitrogen translocation factors may have important effects on protein accumulation in grain. In addition, higher protein concentrations in flag leaves coupled with lower total seed protein of plants peduncle perfused with gibberellic acid indicated that gibberellic acid altered the overall pattern of N allocation within the plant.

Once again, the addition of peduncle N to plants receiving high soil N fertility increased seed protein concentration (Table 2.6), indicating that root uptake of soil N is a limitation to grain N accumulation. The evidence for this is stronger here than in experiment 2 for two reasons. First, whereas four applications of soil fertilizer N, equivalent to 150 kg N ha^{-1}, were made in experiment 2, five applications, equivalent to 187.5 kg N ha^{-1}, were made in experiment 3. Second, the grain protein concentrations and total seed protein levels were not statistically different between experiments 2 and 3 for plants whose only N source was 10.7 mM N, and both values were numerically slightly lower for experiment 3 than experiment 2. Thus, in spite of the 25% increase in soil applied N for plants receiving 10.7 mM soil N and no peduncle N in experiment 3, the concentration and accumulation of grain N did not increase from experiment 2 to experiment 3, indicating that the capacity for root N uptake was saturated at 10.7 mM soil N.

In this study, maximum seed protein concentration, seed protein content and spike protein content were 178.8 mg g^{-1}, 6.1 mg and 354.7 mg, respectively. These maximums were found in plants receiving 10.7 mM N from the soil and a mixture of 30 mM N and GA_3 through the peduncle for seed protein concentration and content

and in plants receiving treatments of 10.7 mM N from the soil and a mixture of 30 mM N and 2,4-D through the peduncle for spike protein content. These increases may be related to the higher grain protein content and seed number found in treatments resulting in the highest seed protein concentration and spike protein content, respectively.

Martin et al. (1990) found that auxin herbicides increased seed protein content when applied at Zadoks growth stage 44. In addition, the plants of experiment 1 perfused with GA_3 or 2,4-D solutions via the peduncle showed higher seed protein content and spike protein content than plants perfused with distilled water. Plants receiving the combination of 30 mM N and GA_3 through the peduncle and 10.7 mM N from soil had an average grain protein concentration of 178.8 mg g^{-1}, 95% higher than plants fertilized and perfused with distilled water. Ma et al. (1994b) found that barley plants perfused with 25 mM N showed the maximum grain protein concentration of 223.8 mg g^{-1}. In experiment 2 maximum accumulated protein was found in plants receiving 10.7 mM N from the soil and 30 mM N through the peduncle.

In general, these results suggest that 1) uptake of N by plant roots is more limiting to seed protein accumulation than is uptake of N containing compounds by seeds, 2) some PGR alter the overall pattern of N allocation between plant parts, although the nature and degree of this alteration is dependent on the amount of N available to the plant, and 3) because the effects of PGR can vary with N level their effects on seed protein are not additive with soil or peduncle N level effects.

Preface to Section 3

Section 3 is material intended for a manuscript by K. Foroutan-pour and D.L. Smith which will be submitted publication as a short paper in the **Journal of Agronomy and Crop Science**. The format has been changed to conform as much as possible with the guidelines set by the Faculty of Graduate Sciences and to be consistent within this thesis. Tables and Figures are presented immediately after they were discussed. References are listed in a separate section at the end of the thesis. In this section we address the overall applicability of the peduncle perfusion technique in the field and the responses of barley grain protein concentration to grain-filling nitrogen and ethephon application through a peduncle perfusion system in the field.

Section 3

FIELD EVALUATION OF THE PEDUNCLE PERFUSION TECHNIQUE

3.1 ABSTRACT

The peduncle perfusion system has been used successfully to evaluate plant responses to post-anthesis plant growth regulators (PGR) and nitrogen applied through the peduncle under greenhouse conditions. No experiment with this technique has been conducted in the field. There are large differences between greenhouse and field environments. Thus, the utility of this technique in the field is still unknown. The objectives of this study were to evaluate i) the overall applicability of the peduncle perfusion technique in the field; and ii) the responses of barley grain protein concentration to grain-filling nitrogen and ethephon application through a peduncle perfusion system under field conditions. The perfusion technique floods the peduncle interior with a selected solution for periods of weeks to months. The plants were allowed to take up urea in two concentrationS (15 and 30 mM N) and ethephon, a PGR, at 15 μ M from the peduncle for 29 days. The total volume of solution taken up by the plants ranged from 35 to 81 mL. Peduncle perfusion with 30 mM nitrogen (urea) increased spike protein concentration and content and grain protein concentration without affecting seed weight spike^{-1}. Peduncle perfusion with ethephon increased grain protein concentration, while spike protein content was not increased over distilled water perfused plants. Using the perfusion technique under field conditions showed that barley feeding through the peduncle could be an appropriate method for studying nutrient translocation or effects of metabolism modifying substances in the field during the grain filling period.

3.2 INTRODUCTION

Plant growth regulators (PGR) are generally applied to reduce crop lodging by reducing crop height. It has been reported that two commonly applied PGR,

chlormequat chloride, [(2-chloroethyl) trimethylammonium chloride] (CCC), and ethephon, [(2-chloroethyl) phosphoric acid] have variable effects on cereal yield (Nafziger et al., 1986, Brown et al., 1973). The lengths of the mainstem apex of barley (Ma et al., 1991) and mainstem panicles of oat (Leitch et al., 1989) were reduced as a result of chlormequat application. Knapp et al. (1988) found chlormequat chloride increased yields but only by reducing lodging. Ethephon application has been shown to increase, not affect and reduce cereal grain yields (Ma et al. 1992a; Green et al. 1986).

Mohamed et al. (1990) found that applications of the PGR ethephon and the fungicide triadimefon both reduced lodging but did not effect yield or grain protein, and sometimes increased tissue N levels. However, grain protein concentration may be increased by ethephon (Morris et al., 1989). It has been concluded that ethephon can influence grain protein concentration by altering both the protein and nonprotein components of the grain (Bulman et al., 1993c). Ma et al. (1994a) examined plant growth regulator effects on protein concentration and yield of spring barley and wheat in the greenhouse. They found PGR (ethephon or chlormequat) application increased grain protein concentration by increasing protein accumulation in the grain.

The peduncle perfusion system has been used successfully to evaluate responses of plants to post-anthesis PGR and nitrogen applied through the peduncle under greenhouse conditions (Ma et al., 1992c, 1994a and 1994b). Ma et al. (1992c, 1994b), using a peduncle perfusion technique, found higher grain nitrogen concentrations in plants perfused with urea nitrogen relative to those perfused with distilled water.

Nitrogen is the most important element in controlling protein concentrations and yields in cereal crops. Grain protein concentration was increased by application of N fertilizer to corn (Zhang et al., 1993; Ippersil et al., 1989), oat (Humphreys et al., 1994; Portch et al., 1968), wheat (Ayoub et al., 1994; Doyle et al., 1991; Gooding et al., 1991; Warder et al., 1963), and barley (Bulman et al., 1993b and 1993a; Birch et al., 1990; Kucey, 1987).

No experiments with this technique have been conducted in the field. There can be large differences between greenhouse and field conditions, particularly for

variables such as tillering, soil moisture, relative humidity, temperature, wind level and damage caused by pests. Variables affecting transpiration rate could strongly affect the amounts of peduncle perfused solutions taken up. Thus, the utility of this technique in the field is still unknown.

The objectives of this study were to evaluate i) the overall applicability of the peduncle perfusion technique in the field; and ii) the responses of barley grain protein concentration to grain-filling nitrogen and ethephon application through a peduncle perfusion system in the field.

3.3 MATERIALS AND METHODS

An experiment was conducted out at the Horticultural Center of the Plant Science Department, McGill University, Ste Anne de Bellevue, QC, Canada. The experiment was planted 17 May 1993 and harvested on 20 August 1993. In the year preceding this experiment, the site was used to produce carrots. The barley seeds were planted in 1 m^2 plots. In this experiment we attempted to supply normal field conditions for the plants. The seeding density was 300 seeds m^{-2} in 10 rows spaced 10 cm apart. The plants received only rainwater, and were fertilized based on a soil test, at 70, 90 and 80 kg ha^{-1} of N, P_2O_5 and K_2O, respectively (CPVQ 1988). Total precipitation, and average monthly maximum and minimum temperatures for May, June, July and August of the experimental year 1993 are given in Table 3.1.

Table 3.1. Precipitation and temperature data for May, June, July and August of 1993 and for the 3-year average.

	Precipitation				Temperature (°C)							
	May	June	July	August	May		June		July		August	
	mm				Max	Min	Max	Min	Max	Min	Max	Min
1993	79.1	74.8	94.8	57.5	18.7	7.9	22.8	12.5	26.3	16.5	26.1	15.2
3-year† Mean	79.2	85.1	113.0	96.4	19.2	7.9	23.4	13.1	25.3	15.2	24.6	15.7

† Mean of 1992, 1991 and 1990 years

The experimental design was a split plot with three blocks. Mainplots were two varieties of barley commonly produced in eastern Canada (Leger and Laurier) and subplots were four peduncle perfusion treatments including 0, 15, and 30 mM N as urea, and ethephon at 15 μ M. The experiment was arranged in a randomized complete block design with three blocks and one replication of each treatment in each block.

A syringe perfusion system was set up when the plants reached ZGS 65 (anthesis). For each main stem, the flag leaf sheath was carefully opened to expose the peduncle for the maximum possible length without injury. Two 26-gauge needles were inserted into the peduncle at a 45° angle. The first needle was approximately 5 cm below the collar and served to allow air to escape during injection; the second needle was approximately 5 to 10 cm below the first and 2 to 10 cm above the flag leaf site of each needle injection. The peduncle surface and the needle were surrounded by a triangle of masking tape to form a cup against the side of the peduncle. The cup was filled with fluid latex (Vultex, General Latex Canada, QC), which dried overnight, sealing the needle to the peduncle.

Solutions were added to the system when the latex was completely dry (after about 48 h). A flexible plastic tubing (Tygon i.d. 0.8, o.d. 2.4 mm) was connected to the needles and fixed with silicone seal. These needles were the standard disposable type (Becton Dickinson, Rutherford, NJ), with the plastic portion removed. A 60-mL syringe barrel fitted with a 21-gauge needle was attached to the tubing leading to the lower needle. This syringe acted as a reservoir of the solution to be tested and was held above the spike. The top of the syringe barrel was covered with a stopper to avoid loss of solution through evaporation and stop insects, etc. from falling into the reservoir. A needle fitted with a small flexible plastic tubing was inserted into a stopper on the top of the syringe, and the end of tubing was held beside the syringe with the opened end facing downward so that it was not blocked by rain water or other materials. The open end of the other tube was held above the top of the syringe barrel and, again, with the opened end turned to face downward.

When solution was added to the syringe barrel, it flowed into the bottom of the peduncle, through the peduncle, and up the tube at the peduncle top. The level of the solution in the syringe barrel reservoir and the upper tube were always the same. The whole system was attached to a 2 cm x 4 cm wooden support stake with a heavy rubber band. One or 2 of the peduncles initially injected showed signs of leakage or blockage. This potential problem was dealt with by equipping more plants than were needed in each plot with the injection apparatus.

Treatments were imposed on 21 July, approximately 3 days after the mainstem heading stage. After a solution was added to the syringe barrel, injected peduncles were examined until maturity to make sure that solution was moving out of the syringe reservoir and that there were no leaks.

In this experiment, data were collected during two phases of plant development: 1) during grain fill (daily amount of absorbed solution, time of maturity) 2) at harvest (total amount of absorbed solution, seed number, grain weight, total seed weight, stem and flag leaf weight, and concentration of N in stem, flag leaf, and seed).

Grains were ground with a Udy cyclone mill (Udy Corp., Fort Collins, CO) fitted with a 1 mm screen. The resulting flour samples were digested with sulphuric acid plus S-type Kjeltab catalyst [Tecator Manual, Kjeltec System 1002 Distilling Unit (Tecator, Höganäs, Sweden)] and the resulting NH_4 was distilled into H_3BO_3 and quantified by titration with dilute acid (AACC, 1983; Bradstreet, 1965). Results were expressed as average weight $seed^{-1}$, and grain N concentration (mg g^{-1}).

The data were analyzed with the GLM procedure of the SAS package (SAS Institute, 1985), according to Steel and Torrie (1980). The amount of solution delivered into a peduncle was used as a covariable for the analysis of average grain weight and grain N concentration. The significance of differences between individual means was determined by a protected least significant difference (LSD) test, ($P \leq 0.05$) analysis.

3.4 RESULTS AND DISCUSSION

This experiment represents the first application of the perfusion technique under field conditions. The technique was successfully utilized under controlled environment conditions by Ma et al. (1992c,1994a and 1994b).

Table 3.2 shows the means ± standard error of solution absorbed. The average solution volume uptake per peduncle over the 29-day feeding period was 65 mL, with a range of 35 to 81 mL. Compared to previous studies (Ma et al., 1992c, 1994a and 1994b), the averaged uptake solution was similar for the same treatments. In this experiment, ranking for volumes of solution uptake was distilled water > ethephon 15 μ M > 15 mM N > 30 mM N. For each plant, lowering the open end of the upper tube to the level of the peduncle resulted in solution dripping from that tube at a rate of several mL min^{-1}, indicating that the variation between plants in rate of solution uptake was not due to restrictions on the rate of solution delivery to the peduncle, but rather to differences in solution absorption by the individual plants. In this experiment the volume of solution absorbed was not a significant covariable for average grain weight and grain protein concentration. Differences in the amount of perfused N and ethephon solutions and distilled water uptake are probably due to the more negative osmotic potentials of the more slowly taken up solutions.

Table 3.2. Total absorption of perfused solution through the peduncle (value are mean ± s.e.).

Treatment	Uptake solution (mL)
30 mM N (Urea)	44.0 ± 2.3
15 mM N (Urea)	67.7 ± 3.0
Ethephon 15 μ M	74.0 ± 2.0
Distilled water	74.3 ± 2.1

Fig 3.1 shows the average daily uptake of solution of plants by treatment. At the beginning of grain-filling absorption of solution was maximal. This was followed by an approximately linear decline of -0.14 mL d^{-1}. One explanation for the decline could be that during grain-filling the transpiration rate of most tissues, and especially the flag leaf and spike, are high (Simpson et al., 1983; Stoskopf, 1985). But, as the

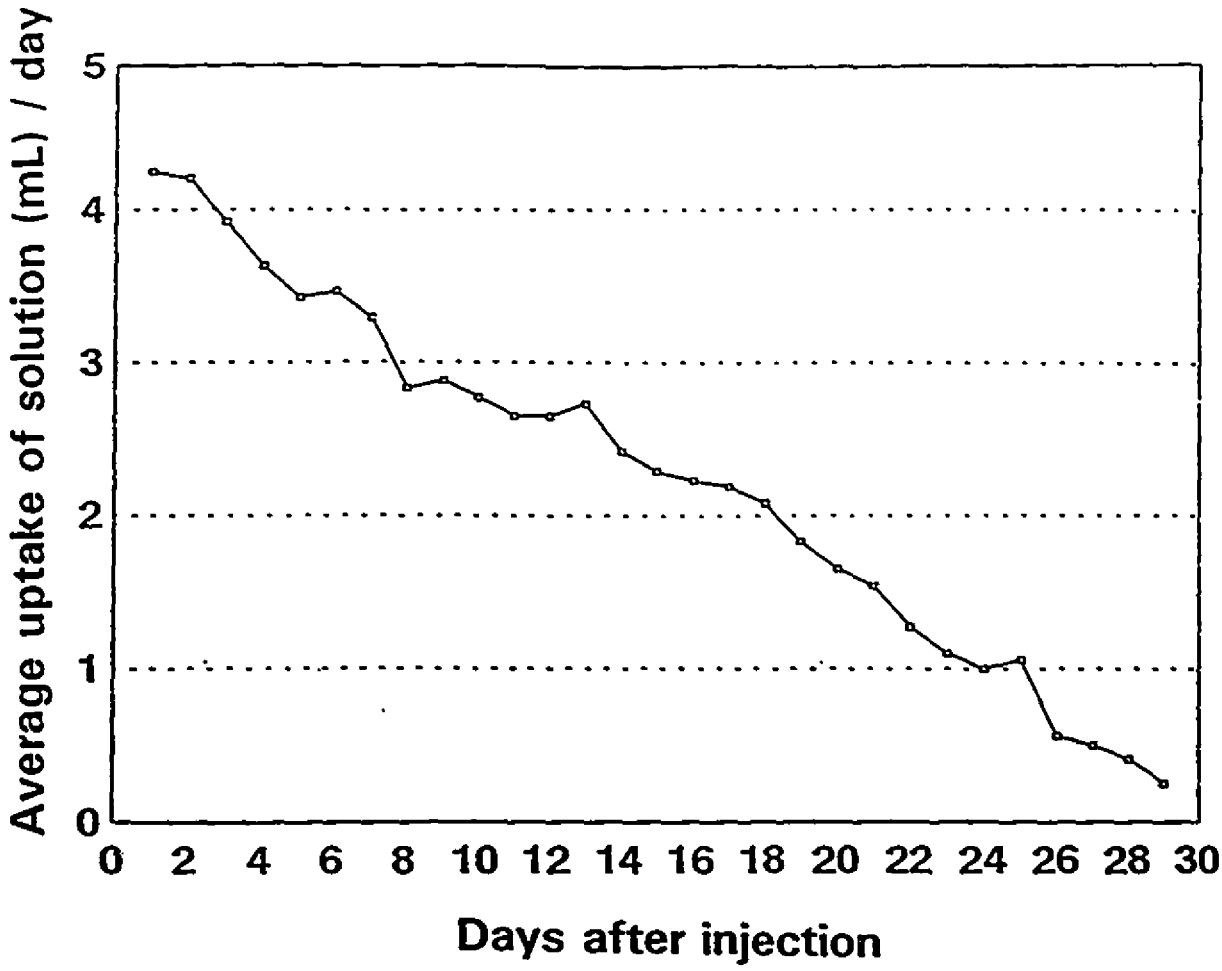

Fig. 3. 1. Average daily uptake of solution for all plants from the 1st to 29th day after injection.

plants mature transpiration losses decrease.

Peduncle perfusion with N or ethephon solutions did not affect seed number and seed weight per spike when compared to plants perfused with distilled water, with the exception of seed number produced by plants perfused with 30 mM N which was more than plants perfused with distilled water (Table 3.3). Ma et al. (1994b) found no difference for seed weight per spike between plants perfused with N or ethephon and distilled water, but differences were found for seed number.

Fertilization and the beginning of grain formation happen before spike emergence, while the perfusion system is attached after spike emergence. Thus it might be expected that the treatments would not affect the number of florets, and therefore, potential seeds established. The level of floral abortion could be affected by the applied treatments, but this was not the case (Table 3.3).

There were no differences between treatments for weight per seed (Table 3.3). The average weight seed^{-1} was quite high (42.5 mg). Ma et al. (1992c, 1994a and 1994b) found no difference for weight per seed among greenhouse grown plants perfused with ethephon, 30 mM N or distilled water. We did find a significant difference in weight and protein content per seed between the two barley varieties. Average grain weight and protein content per seed of Laurier was significantly higher than that of Leger (data not shown). Laurier was found to have the highest weight per seed among twenty six Québec grown barley cultivars (Bulman et al., 1993d).

Our data showed that peduncle perfusion with 30 mM nitrogen increased total spike protein content without affecting seed weight spike^{-1} or weight seed^{-1}. The increase was due to an increase in protein concentration (Table 3.3). Non-significant increases in seed protein content and weight per seed were noted. These non-significant differences must have been at least partly real as they appear to have produced a significant protein concentration difference. Increased nitrogen concentration by perfusion with a nitrogen solution was reported under controlled environment conditions by Ma et al. (1992c and 1994d).

Peduncle perfusion with ethephon increased grain protein concentration by 6% compared to distilled water. Ma et al. (1994a) found peduncle perfusion with ethephon increased grain protein concentration by 13% compared to distilled water.

Table 3.3. Mean seed number, seed weight produced by the spike of the infused stem, grain protein concentration, spike protein content, grain weight per seed, grain protein content, flag leaf weight, flag leaf protein concentration, mainstem weight, and mainstem protein concentration.

Treatment		Measurement+									
Variety	Treatment+	SN	GPC -mg g^{-1}-	SW --g--	SPC --mg--	WS -mg-	PCS -mg-	FW --g--	FPC -mg g^{-1}-	MW --g--	MPC -mg g^{-1}-
Leger	N30	55	171.2	2.1	363.8	38.6	6.6	0.22	162.9	0.71	50.4
	N15	53	155.8	2.1	329.4	39.9	6.2	0.15	128.3	0.71	43.6
	E	51	164.3	2.1	341.6	40.5	6.7	0.20	106.9	0.81	51.9
	DW	51	150.5	2.2	325.5	42.8	6.4	0.21	70.8	0.81	47.9
Laurier	N30	49	176.5	2.2	384.0	44.1	7.8	0.22	166.7	0.79	47.2
	N15	49	160.5	2.2	351.6	44.9	7.2	0.18	122.8	0.79	44.3
	E	51	158.0	2.2	343.8	43.0	6.8	0.20	99.5	0.77	54.7
	DW	49	152.8	2.3	344.7	46.4	7.1	0.15	82.2	0.78	41.3
LSD$_a$		2.8	13.2	0.2	35.6	4.7	0.8	0.03	16.7	0.1	5.9
LSD$_b$		3.4	1.4	0.2	34.3	4.3	0.8	0.04	23.1	0.1	9.2
Variety		*	NS	**	NS	**	*	NS	NS	NS	NS
Treatment		NS	**	NS	*	NS	NS	***	***	NS	***
Variety X Treatment		NS	NS	NS	NS	NS	NS	**	NS	NS	NS
C.V.$^{\dashv}$		3.1	4.6	5.4	5.8	6.2	6.5	8.7	7.9	6.8	6.9

LSD^a Least significant difference for comparison of treatments within the varieties ($P \leq 0.5$)
LSD^b Least significant difference for comparison of treatments across the varieties ($P \leq 0.5$)
NS, *, **, or *** For the overall model; no significant difference or different at the 0.05, 0.01 and 0.001 level of probability, respectively.
+ Treatment notation: N30 = 30 mM N as urea; N15 = 15 mM N as urea; E = 15 μ M ethephon; DW = distilled water.
+ Measurement notation: SN = seed number; GPC = grain protein concentration; SW = seed weight per spike; SPC = spike protein content; WS = weight per seed; PCS = protein content per seed; FW = flag leaf weight of mainstem; FPC = flag leaf protein concentration of mainstem; MW = mainstem weight; MPC = mainstem protein concentration
$\dashv$ Coefficient Variation (%)

A field study (Mohamed et al., 1990) and a greenhouse study (Ma et al., 1994b) regarding application effects of ethephon on grain protein concentration demonstrated that application of ethephon did not affect grain protein concentration. Conversely, ethephon application by peduncle perfusion under greenhouse conditions (Ma et al., 1994a) and ethephon spray under field conditions (Ma et al., 1994a; Morris et al., 1989) increased grain protein concentration by increasing protein accumulation in the grain, although, protein yield increases were small because of grain yield reduction.

Compared to peduncle perfusion with distilled water, peduncle perfusion with the nitrogen treatments of 15 and 30 mM N and peduncle perfusion with ethephon significantly increased flag leaf protein concentration (Table 3.3). In addition, there was a difference in flag leaf protein concentration between plants peduncle perfused with 30 mM N and 15 mM N or ethephon. Similarly, Ma et al., (1994b) found higher nitrogen and ^{15}N concentrations in the chaff and in flag leaves in N-treated greenhouse grown plants when compared to water-perfused controls.

Higher protein concentrations in both grain and flag leaves of plants peduncle perfused with 30 mM N and ethephon (Table 3.3) suggest that when there is not a nitrogen sink limitation for field produced barley when supplied with N through the peduncle. Ma et al. (1994b) found higher N and ^{15}N concentrations in the flag leaves of greenhouse grown plants treated with N solutions when compared to plants treated with distilled water. Nitrogen translocation factors may have important effects on protein accumulation in grain. Moreover differences in flag leaf protein concentration of plants peduncle perfused with 15 mM N relative to plants peduncle perfused with distilled water, but without differences in grain protein concentration or spike protein concentration (Table 3.3) suggest that processes related to nitrogen translocation into the grain are important once the accumulation of nitrogen in those tissues has approached an upper limit. Higher mainstem protein concentrations along with increases, or no change in seed protein concentration in plants treated with ethephon than other treatments indicated that ethephon stimulated protein synthesis processes.

Generally, experiments conducted under field and greenhouse conditions produced similar results. According to several studies, including the present one, ethephon has the potential to increase the concentration of grain protein without decreasing the weight per seed. This suggests that ethephon application could be utilized when higher protein yield than grain yield is desired. This subject needs more consideration under both field and greenhouse conditions.

GENERAL DISCUSSION

In this study, the peduncle perfusion technique developed by Ma and Smith (1992c) was successfully used to deliver various substances to barley plants during the grain filling period under controlled environment and field conditions (Sections 2, 3). Application of this technique under field conditions (Section 3) indicated that barley feeding through the peduncle could be an appropriate method for studying nutrient translocation or effects of metabolism modifying substances in the field during the grain filling period. The technique was successfully utilized under controlled environment conditions by Ma et al. (1992c, 1994a and 1994b).

The volume of solution absorbed by plants was not usually a significant covariable in the statistical models even of were observed big difference for average weight, protein concentration, and protein content per seed. Differences in the uptake volume between the perfused solutions are probably due to the greater osmotic potential of the more concentrated solutions. Trends for daily uptake of solution by plants from heading to maturity showed an approximately linear decline (Section 2, 3). This could be because of the high transpiration rate of most tissues, and especially the flag leaf and spike (Simpson et al., 1983; Stoskopf, 1985) during the grain-filling.

Availability of N sources to barley plants during the grain-filling can alter the amount of seed protein concentration and seed protein content. Plants fertilized well with N fertilizer had higher protein content and concentration per seed (Section 2). Grain protein concentration was increased by application of N fertilizer to barley (Bulman et al., 1993b and 1993a; Birch et al., 1990; Kucey, 1987). In addition, as Ma et al. (1992c, 1994b) found plants receiving N solution through the peduncle appeared to have high seed protein concentration and seed protein content. Weight per seed was generally not affected by N applied in the soil or in the peduncle. No difference for weight per seed of plants perfused with various N concentrations was reported by Ma et al. (1992c, 1994b). Consequently increasing seed protein concentration but unchanging weight per seed in plants as affected by soil N or peduncle N indicated that more available N during the grain-filling increased the protein / carbohydrate ratio.

The results of experiments 1 and 3 (Section 2) showed that accumulation of protein in the grain of plants receiving GA_3 or 2,4-D in experiment 1 and a mixture of 30 mM N and GA_3 in experiment 3 through the peduncle was higher than that of plants receiving distilled water. These increases may be related to the higher grain protein content found in these treatments, resulting in the higher seed protein concentration. Gibberellin induces the formation of α-amylase by increasing Ca^{+2} flux into the endoplasmic reticulum in the aleurone layer of barley seeds (Bush et al., 1993; Hewitt et al., 1968). The maximum of spike protein content was found in plants receiving treatment of 10.7 mM N from the soil and a mixture of 30 mM N and 2,4-D through the peduncle (Section 2). This could be because of higher seed number or seed weight $spike^{-1}$ found in this treatment, resulting in the highest spike protein content. It was surprising that 2,4-D decreased seed weight per spike in experiment 1, while, 2,4-D increased seed weight per spike in experiment 3. However, the 2,4-D effects observed in experiment 3 occurred only at the highest N levels, levels that were not used in experiment 1. This may indicate that some of the potential PGR effects will only be manifested under specific conditions. Several studies indicated that auxin herbicides increased seed protein and starch content (Martin et al., 1990; Bangerth et al., 1985; Rademacher et al., 1984; Hewitt et al., 1968).

Higher protein concentrations in grain, flag leaves and mainstems of plants peduncle perfused with 30 mM N and receiving 10.7 mM N soil suggests that there is no nitrogen sink limitation when the plant receives additional N from the peduncle. Similar results were found in experiment 2 (Section 2) and by Ma et al. (1994b). Nitrogen translocation factors may have important effects on protein accumulation in grain. In addition, higher protein concentrations in both grain and flag leaves of plants peduncle perfused with gibberellic acid indicated that gibberellic acid stimulated some aspect of protein synthesis.

Ethephon application by peduncle perfusion under field conditions (Section 3) increased grain protein concentration by increasing protein content in grain. A similar result was found by Ma et al. (1994a) under greenhouse conditions. Thus, results obtained under field and greenhouse conditions indicated that ethephon has the

potential to increase the concentration of grain protein without decreasing the weight seed^{-1}. This suggests that ethephon application could be utilized when higher protein yield than grain yield is desired. This subject needs more consideration under both field and greenhouse conditions.

In general, the results presented in this study (Section 2 and 3) indicated that availability of different N sources during the vegetative growth and grain filling stages, and probably stimulation of some protein synthesis processes caused by PGR may lead to accumulation of more protein in grain. More consideration of correlations between plant hormones and grain protein synthesis is required.

SUMMERY OF RESULTS

1. Protein concentration and content per seed of plants receiving 2,4-D and gibberellic acid were highest and those of plants receiving ABA were lowest.
2. The frequency and degree of PGR effects were greatest for the top third of the spike.
3. Kinetin and abscisic acid treatments generally resulted in the highest and lowest levels, respectively, for flag leaf net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration.
4. For experiment 2, both seed protein content spike^{-1} and seed protein concentration in plants fertilized with 10.7 mM N via the soil and plants perfused with 30 mM N via the peduncle were higher than plant received distilled water.
5. For experiment 3, plants receiving only 10.7 mM N from the soil and mixture of 30 mM N and GA_3 or 2,4-D through the peduncle had increased protein content per seed, and the highest total seed weight produced by the perfused stem, respectively.
6. Peduncle perfusion with ethephon increased grain protein concentration, while spike protein content was not increased over distilled water perfused plants.
7. The processes related to nitrogen translocation from tissues to the grain are important once the accumulation of nitrogen in those tissues has approached an upper limit.
8. Using the perfusion technique under field conditions showed that barley feeding through the peduncle could be an appropriate method for studying nutrient translocation or effects of metabolism modifying substances in the field during the grain filling period.

ACCEPTANCE OR REJECTION OF HYPOTHESISES

1) The roots are a bottle-neck for N entry into cereal plants. **Accept, see last paragraph of section 2.**

2) The addition of some plant growth regulators will substantially alter the distribution of N among plant parts. **Accept or reject depends on condition, see last paragraph of section 2.**

3) The stimulatory effects of peduncle perfused PGR and N (urea), and soil N on seed protein concentration are additive. **Reject, see last paragraph of section 2.**

4) The peduncle perfusion technique can be applied to field grown plants and will produce essentially the same results as have already been observed in controlled environment produced plants. **Accept, see last paragraph of section 3.**

LITERATURE CITED

American Association of Cereal Chemists. 1983. Modified Kjeldahl-boric Acid Method 46-12. pp.1-3. In Approved Methods of the American Association of Cereal Chemists. Vol. 2. AACC, St. Paul, MN.

Austin R.B., Flavell R.B., Henson I.E. and Lowe H.J.B. 1986. Molecular Biology and Crop Improvement. Cambridge University Press.

Austin R.B., Ford M.A. and Edrich J.A. 1977a. The Nitrogen Economy of Winter Wheat. J. Agric Sci Camb. 88:159-167.

Austin R.B., Edrich J.A., Ford M.A. and Blackwell R.D. 1977b. The Fate of the Dry Matter, Carbohydrates and ^{14}C Lost From the Leaves and Stems of Wheat During Grainfilling. Annals of Botany. 41: 1309-1321.

Ayoub M., Guertin S. and Smith D.L. 1994. Nitrogen Fertilizer Rate and Timing Effect on Bread Wheat Protein in Eastern Canada. J. Agron. Crop Sci. (Submitted)

Ballantyne A.K. (1962). Tolerance of Cereal Crops to Saline Soils in Saskatchewan. Can. J. Soil Sci. 42: 61-67.

Bangerth F., Aufhammer W. and Baum O. 1985. IAA Level and Dry Matter Accumulation at Different Positions Within a Wheat Ear. Physiologia Plantarum 63: 121-125.

Barley K.P. and Naidu N.A. 1964. The Performance of Three Australian Wheat Varieties at High Levels of Nitrogen Supply. Aust. J. Exp. Agric. Anim. Husb. 4: 39-48.

Barlow E. W. R., Donovan G. R. and Lee J. W. 1983. Water Relations and Composition of Wheat Ears Grown in Liquid Culture: Effect of Carbon and Nitrogen. Aust. J. Plant Physiol., 10: 99-108.

Beevers L. and Hageman R.H. 1969. Nitrate Reduction in Higher Plants. Annu Rev Plant Physiol. 20: 495-522.

Beevers L., Hageman R.H. 1980. Nitrate and Nitrite Reduction. In PK Stumpf, EE Conn, eds, The Biochemistry of Plants, Vol 5. BJ Miflin, ed, Amono Acids and Derivatives. Academic Press, New York, pp. 115-164.

Bell C. J. and Incoll L. D. 1990. The Redistribution of Assimilate in Field-grown Winter Wheat. J. of Exp. Bot. 41: 949-960.

Bidinger F., Musgrave R.B. and Fischer R.A. 1977. Contribution of Stored Pre-anthesis Assimilate to Grain Yield in Wheat and Braley. Nature (London). 270: 431-433.

Birch C.J. and Long K.E. 1990. Effect of Nitrogen on the Growth, Yield and Grain Protein Content of Barley (Hordeum Vulgare). Aust. J. Exp. Agric. 30: 237-242.

Bonnett G. D. and Incoll L. D. 1992. The Potential Pre-anthesis and Post-anthesis Contributions of Stem Internodes to Grain Yield in Crops of Winter Barley. Ann. Bot. 69: 219-225.

Borrell A.K., Incoll L.D., Simpson R.J. and Dalling M.I. 1989. Partitioning of Dry Matter and the Deposition and Use of Stem Reserves in a Semi-dwarf Wheat Crop. Annals of Botany. 63: 527-539.

Bousquet J.F., Touraud G., Piollat M.T., Bosch U. and Trottet M. 1990. ABA Accumulation in Wheat Heads Inoculated With Septoria Nodorum in the Field Conditions. Journal of Agronomy and Crop Science 165: 297-300.

Bradstreet R.B. 1965. The Kjeldahi Method for Organic Nitrogen. Academic Press, New York.

Brown C.M. and Earley E.B. 1973. Response of one Winter Wheat and Two Spring Oat Varieties to Faliar Applications of 2-chloroethyl Phosphonic Acid (Ethrel). Agron. J. 65: 829-832.

Brun W.A., Brenner M.L. and Schussler J. 1985. Hormonal Communication Between Sources and Sinks in Soybeans. InJeffcoat B., Hawkins A.F. and Stead A.D. (eds). Regulation of Sources and Sinks in Crop Plants. Monograph 12, British Plant Growth Regulator Group, Bristol.

Bulman P. and Smith D.L. 1993a. Accumulation and Redistribution of Dry Matter and Nitrogen by Spring Barley. Agronomy J. 85: 1114-1121.

Bulman P. and Smith D.L. 1993b. Grain Protein Response of Spring Barley to High Rates and Post-Anthesis Application of Fertilizer Nitrogen. Agronomy J. 85: 1109-1113.

Bulman P. and Smith D.L. 1993c. Yield and Grain Protein Response of Spring Barley to Ethephon and Triadimefon. Crop Science 33: 798-803.

Bulman P., Dian E. Mather, and Donald L. Smith. 1993d. Genetic Improvement of Spring Barley Cultivars Grown in Eastern Canada From 1910 to 1988. Euphytica 71: 35-48.

Bush D.S., Biswas A.K. and Jones R.L. 1993. Hormonal Regulation of Ca^{2+} Transport in the Endomembrane System of the Barley Aleurone. Planta. Berlin : Springer-Verlag. 189: 507-515.

Clifford P.E., Pentland B.S. and Baylis A.D. 1992. Effects of Growth Regulators on Reproductive Abscission in Faba Bean (Vicia faba cv. Troy). Journal of Agricultural Science 119: 71-78.

Corke H. and Atsmon D. 1990. Endosperm Protein Accumulation in Wild and Cultivated Barley and Their Cross Grown in Spike Culture. Euphytica. 48: 225-231.

Corke H. and Atsmon D. 1988. Effect of Nitrogen Nutrition on Endosperm Protein Synthesis in Wild and Cultivated Barley Grown in Spike Culture. Plant Physiol. 87: 523-528.

Corke H., Avivi N. and Atsmon D. 1989. Pre- and Post-anthesis Accumulation of Dry Matter and Nitrogen in Wild Barley (Hordeum spontaneum) and in Barley Cultivars (H. vulgare) Differing in Final Grain Size and Protein Content. Euphytica 40: 127-134.

Croy, L.I. and R.H. Hageman 1970. Relationship of Nitrate Reductase Activity to Grain Protein Production in Wheat. Crop Sci. 10: 280-285.

Crowder M.J. and Hand D.J. 1990. Analysis of Repeated Measures. Chapman and Hall.

Dalling, M.J., G. Boland and J.H. Wilson 1976. Relation between Acid Proteinase Activity and Redistribution of Nitrogen during Grain Development in Wheat. Aust. J. Plant Physiol. 3: 721-730.

Dalling, M.J., G.M. Halloran and J.H. Wilson 1975. The Relation between Nitrate Reductase Activity and Grain Nitrogen Productivity in Wheat. Aust. J. Agric. Res. 26: 1-10.

Dalling, M.J. 1985. The Physiological Basis of Nitrogen Redistribution During Grain Filling in Cereals. In: Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity. (Eds. J.E. Harper, L.E. Schrader, and R.W. Howell). pp. 55-69. American Society of Plant Physiologists.

Davies W. J. and Mansfield T. A. 1987. Auxins and stomata. In: E. Zeiger, G. D. Farquhar and I.R. Cowan (Editors), Stomatal Function. Stanford University Press, Stanford, CA, pp. 293-309.

Deckard, E.L., R.J. Lambert, and R.H. Hageman 1973. Nitrate Reductase Activity in Corn Leaves as Related to Yield of Grain and Grain Protein. Crop Sci. 13: 343.

Deckerd, E.L., R.H. Busch, and K.D. Kofoid 1985. Physiological Aspects of Spring Wheat Improvement. In: Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity. (Eds. J.E. Harper, L.E. Schrader, and R.W. Howell). pp. 46-53. American Society of Plant Physiologists.

Desai R. M. and Bhatia C. R. 1978. Nitrogen Uptake and Nitrogen Harvest Index in Durum Wheat Cultivars Varying in Their Grain Protein Concentration. Euphytica 27: 561-566.

Dodge C. S. and Hiatt A. J. 1972. Relationship of pH to Ion Uptake Imbalance by Varieties of Wheat (Triticum vulgare). Agron. J. 64: 476-481.

Doll H. and Andersen B. 1981. Preparation of Barley Storage Protein, Hordein, for Analytical Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis. Anal. Biochem. 115: 61-66.

Doyle A.D. and Shapland R.A. 1991. Effect of Split Application on the Yiel and Protein Content of Dryland Wheat in Nothern New South Wales. Aust. J. Exp. Agric. 31:85-92.

Duffield R.D. 1971. Master's Thesis, Oklahoma State University, Still-water, Okla.

Eilrich G.L. and Hageman R.H. 1973. Nitrate Reductase Activity and its Relationship to Accumulation of Vegetative and Grain Nitrogen in Wheat (Triticum aestivum L.). Crop Sci. 13:

Ellis E.C., Turgeon R. and Spanswick R.M. 1992. Quantitative Analysis of Photosynthate Unloading in Developing Seeds of Phaseolus vulgaris L. II. Pathway and Turgor Sensivity. Plant Physiol. Rockville, Md. : American Society of Plant Physiologists. Vol: 99 (2) p. 643-651. 59-65.

Fisher D.B. and Frame J.M. 1984. A Guide to the Use of the Exuding Stylet Technique in Phloem Physiology. Planta. 161: 385-393.

Fisher D.B. and Gifford R.M. 1987. Accumulation and Conversion of Sugars By Developing Wheat Grains. VII. Effects of Changes in Tube and Endosperm Cavity Sap Concentration on the Grain Filling Rate. Plant Physiol. In Press.

Fisher B. D. and P. K. Macnicol 1986a. Amino Acid Composition Along the Transport Pathway during Grain Filling in Wheat. Plant Phsiol. 82: 1019-1023.

Fisher B. D. and R. M. Gifford 1986b. Accumulation and Conversion of Sugars by Developing Wheat Grains. Plant Physiol. 82: 1024-1029.

Frith, G.J.T., D.G. Bruce and M.J. Dalling 1975. Distribution of Acid Proteinase Activity in Wheat Seedlings. Plant and Cell Physiol. 16: 1085-1091.

Gallagher J.N., Biscoe P.V. and Hunter B. 1976. Effects of Drought on Grain Growth. Nature. 264: 541-542.

Gallagher R.N., Ashley D.A. and Brown R.H. 1975. ^{14}C-photosynthate Translocation in C_3 and C_4 plants as Related to Leaf Anatomy. Crop Sci. 15: 55-59.

Giese H., Andersen B. and Doll H. 1983. Synthesis of the Major Storage Protein, Hordein, in Barley. Planta. 159: 60-65.

Gooding M.J., Kettlewell P.S. and Hocking T.J. 1991. Effects of Urea Alone or With Fungicide on the Yield and Breadmaking Quality of Wheat When Sprayed at Flag Leaf and Ear Emergence. J. Agric Sci. (Cambrige) 117: 149-155.

Gouvernement du Guebec, Ministere de L'Agriculture 1988. Cereales De Printemps Culture. Agdex 110/20.

Grabau, L.J., D. G. Blevins, and H. C. Minor 1986. Stem Infusions Enhanced Methionine Content of Soybean Storage Protein. Plant Physiol. 82: 1013-1018.

Green C.F. and Dawkins T.C.K. 1986. Influence of Nitrogen Fertilizer and Chlormequat on Two Spring Wheat Cultivars. Crop-Res. Edinburgh : Scottish Academic Press. V. 25 (2) p. 89-101.

Gregory P.J., Marshall B. and Biscoe P.V. 1981. Nutrient Relations of Winter Wheat. 3. Nitrogen Uptake, Photosynthesis of Flag Leaves and Translocation of Nitrogen to Grain. J. Agric. Sci. 96: 539-547.

Guinn G. and Brummett D.L. 1993. Leaf Age, Decline in Photosynthesis, and Changes in Abscisic Acid, Indole-3-acetic Acid, and Cytokinin in Cotton Leaves. Field Crops Research 32: 269-275.

Gupta, S.C. and L. Beevers 1985. Regulation of nitrate reduction. In: Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity. (Eds. J.E. Harper, L.E. Schrader, and R.W. Howell). pp. 1-10. American Society of Plant Physiologists.

Hardy P.J. 1969. Selective Diffusion of Basic and Acidic Products of CO_2-fixation into the Transpiration Stream in Grapevine. J. Exp. Bot. 20: 856-862.

Harper J.E. and Paulsen G.M. 1967. Changes in Reduction and Assimilation of Nitrogen During the Growth Cycle of Winter Wheat. Crop Sci. 7: 205-209.

Haunold A., Johnson V.A. and Schmidt J.W. 1962. Variation in Protein Content of the Grain in Four Varieties of Triticum aestivum L. Agron. J. 54: 121-125.

Herzong H. 1982. Relation of Source and Sink During Grain Filling Period in Wheat and Some Aspects of its Regulation. Physiologia Plantarum 56: 155-160.

Hewitt E.J. and Cutting C.V. 1968. Recent Aspects of Nitrogen Metabolism in Plants. pp. 189-199.

Humphreys DG., Smith D.L. and Mather DE. 1994. Nitrogen Fertilizer and Seeding Date Induced Changes in Protein, Oil and ß-glucan Contents of Four Oat Cultivars. J. Cereal Sci. (Submitted)

Incoll L. D. and Jewer P. C. 1987. Cytokinins and stomata. In: E. Zeiger, G. D. Farquhar and I.R. Cowan (Editors), Stomatal Function. Stanford University Press, Stanford, CA, pp. 281-292.

International Rice Research Institute. 1972. Annual Report for 1971. Los Bafios, Philippines. pp. 11-14.

Ippersiel D., Alli I., Mackenzie A.F. and Mehuys G.R. 1989. Nitrogen Distribution, Yield, and Quality of Silage Corn After Foliar Nitrogen Fertilization. Agron. J. 81: 783-786.

Ishizuka Y. and Tranaka A. 1953. Biochemical Studies on the Life History of Rice Plants. 3. Synthesis and Translocation of Nitrogen Compounds and Carbohydrates. Nippon Dojo-Hiryogaku Zasshi. 23: 159-165. (English Translation (Typescript)).

Johnson V.A., Schmidt J.W. and Mattern P.J. 1968. Cereal Breeding For Better Protein Impact. Econ. Bot. 22: 16-25.

Kaufman P.B. and Dayanandan P. 1983. Gibberellin-induced Growth in Avena Internodes. In: Crozier, A. (ed.), The Biochemistry and Physiology of Gibberellins, Vol. II, Praeger, New York, USA.

Kirby E.J.M. and Faris D.G. 1970. Plant Population Induced Growth Correlations in the Barley Plant Main Shoot and Possible Hormonal Mechanisms. Journal of Experimental Botany. 21: 787-798.

Knapp J.S. and Harms C.L. 1988. Nitrogen Fertilization and Plant Growth Regulator Effects on Yield and Quality of Four Wheat Cultivars. J-Prod-Agric. Madison, Wis. : American Society of Agronomy. V. 1 (2) p. 94-98.

Koehler S.M. and Ho T.H.D. 1990. Hormonal Regulation, Processing, and Secretion of Cysteine Proteinases in Barley Aleurone Layers. Plant-Cell. Rockville, Md. : American Society of Plant Physiologists. 2: 769-783.

Koshkin E.I. and Tararina V.V. 1990. Differences in Source-Sink Ratios in Wheat and Their Relationship to Gain Yield and Content of Abscisic Acid. Plant Physiology and Biochemistry 28: 609-615.

Kreis, M. and P.R. Shewry 1992. The Control of Protein Synthesis in Developing Barley Seeds. In: Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. (Ed. P.R. Shewry). pp. 319-330. C.A.B. International.

Kucey R.M.N. 1987. Nitrogen Fertilizer Application Practices for Barley Production Under Southwestern Canadian Prairie Cnditions. Commun. Soil Sci. Plant Anal. 18:753-769.

Kuhbauch W. and Thome U. 1989. Nonstructural Carbohydrates of Wheat Stems as Influenced By Sink-source Manipulations. Journal of Plant Physiology. 134: 243-250.

Leitch M. H. and Hayes J. D. 1989. Effects of Chlormequat Application on Stem Characteristics, Yield and Panicle Conformation of Winter Oats. J. of Agricultural Sci., Cambridge. 113: 17-26.

Levitt J. 1972. Responses of Plants to Environmental Stresses. Academic Press. pp. 489-530.

Ma, B.L. and D.L. Smith 1991. Apical Development of Spring Barley in Relation to Chlormequat and Ethephon. Agron. J. 83:270-274.

Ma B. L., Leibovitch S., Maloba W.E. and Smith D.L. 1992a. Spring Barley Responses to Nitrogen Fertilizer and Ethephon in Regions with a Short Crop Growing Season. J. Agronomy and Crop Science. 169: 151-160.

Ma, B.L. and D.L. Smith 1992b. Post-Anthesis Ethephon Effects on Yield of Spring Barley. Agron. J. 84:370-374.

Ma, B.L. and D.L. Smith. 1992c. New Method for Supplying Substances to Cereal Inflorescences. Crop Sci. 32: 191-194.

Ma, B.L. and D.L. Smith 1992d. Growth Regulator Effects on Aboveground Dry Matter Partitioning during Grain Fill of Spring Barley. Crop Sci. 32: 741-743.

Ma B.L., Leibovitch S. and Smith D.L. 1994a. Plant Growth Regulator Effects on Protein Cotent and Yield of Spring Barley and Wheat. J. Agronomy and Crop Sci. 172: 9-18.

Ma , B.L. 1994b. Carbon Metabolism and Nitrogen-15 Distribution of Barley and Wheat Following Peduncle-added Nitrogen and Growth Regulators. In press.

Macisaac S.A. and Sawhney V.K. 1990. Protein Changes Associated With Auxin-induced Stimulation and Kinetin-induced Inhibition of Lateral Root Initiation in Lettuce (Lactuca sativa) Roots. J-Exp-Bot. Oxford : Oxford University Press. 41: 1039-1044.

MacKown C. T. and Van Sanford D. A. 1986. In situ Nitrate Assimilation in Winter Wheat: Peduncle Injection with Nitrogen-15-Nitrate at Anthesis. Agron. J. 78: 311-317.

Martin P. 1982. Stem Xylem as a Possible Pathway for Mineral Retranslocation from Senescing Leaves to the Ear in Wheat. J. Plant Physiol. 9: 197-207.

Martin D.A., Miller S.D. and Alley H.P. 1990. Spring Wheat Response to Herbicides Applied at Three Growth Stages. Agron-J. Madison, Wis. : American Society of Agronomy. 82: 95-97.

Mather D. E. and Poysa V. W. 1983a. Genetic Analysis of the Protein and Lysine Content of Spring Triticale. Can. J. Genet. Cytol. 25: 378-383.

Mather D. E. and Poysa V. W. 1983b. Griffing and Hayman Diallel Analyses of Protein and Lysine Content of Spring Triticale. Can. J. Genet. Cytol. 25: 384-389.

Mather D. E. and Giese H. 1984. Protein and Carbohydrate Accumulation in Normal and High-lysine Barley in Spike Culture. Physiol. Plant. 60: 75-80.

McConnell W.B. and Ramachandran L. K. 1956. Acetate Metabolism of Maturing Wheat Plants. Can. J. of Biochem. and Physiol. 34: 180-190.

McDonnell, E. and R.G. Wyn Jones. 1988. Glycinebetaine Biosynthesis and Accumulation in Unstressed and Salt-stressed Wheat. J. Exp. Bot. 39:421-30.

McNeal F.H., Berg M.A. and Watson C.A. 1966. Nitrogen and Dry Matter in Five Spring Wheat Varieties at Successive Stages of Development. Agron. J. 58: 605-608.

Michael G. and Seiler-Kelbitsch H. 1972. Cytokinin Content and Kernel Size of Barley Grain as Affected by Environmental and Genetic Factors. Crop Science 12: 162-165.

Miflin B.J. and Lea P.J. 1980. Ammonia Assimilation. In BJ Miflin, ed, The Biochemistry of Plants, Vol 5. Academic Press, New York, pp. 169-202.

Mohamed M.A., Steiner J.J., Wright S.D., Bhangoo M.S. and Millhouse D.E. 1990. Intensive Crop Management Practices on Wheat Yield and Quality. Agronomy J. 82: 701-706.

Morris D.A. 1982. Hormonal Regulation of Sink Invertase Activity: Implications for the Control of Assimilate Partitioning. In Wareing P F (ed) Plant Growth Substances 1982. Academic Press.

Morris D.A. 1983. Hormonal Regulation of Assimilate Partition: Possible Mediation by Invertase. News Bulletin of the British Plant Growth Regulator Group 6: 23-35.

Morris C.F., Ferguson D.L. and Paulsen G.M. 1989. Nitrogen Fertilizer Management With Foliar Fungicide and Growth Regulator for Hard Winter Wheat Production. Appl. Agric. Res. 4: 135-140.

Nafziger E.D., Wax L.M. and Brown C.M. 1986. Response of Five Winter Wheat Cultivars to Growth Regulators and Increased Nitrogen. Crop Sci. 26: 767-770.

Nair T.V.R. and Abrol Y.P. 1977. Studies on Nitrate Reducing System in Developing Wheat Ears. Crop Sci. 17: 438-442.

Nevo E., Atsmon D. and Beiles A. 1985. Protein Resources in Wild Barley, H. spontaneum, in Israel: Predictive Method by Ecology and Allozyme Markers. Plant Syst. Evol. 150: 205-222.

Oritant T. and Yoshida R. 1971. Studies on Nitrogen Metabolism in Crop Plants. XI. The Changes in Abscisic Acid Cytokinin-like Activity Accompanying With Growth and Senescence in the Crop Plants. Proc. Crop Sci. Soc. Japan 40: 325-331.

Osborne, T.B. 1924. The Vegetable Proteins. Longman, Green and Co.,London, 154 pp.

Pate J.S. 1975. Exchange of Solutes Between Phloem and Xylem and Circulation in the Whole Plant. In 'Transport in Plants. I. Phloem Transport'. (Eds M. H. Zimmermann and J.A. Milburn.) Encycl. Plant Physiol. New Series, Vol. 1, pp.453-473. (Spring-Verlag: Heidelberg.).

Perez, C.M., G.B. Cagampang, B.V. Esmama, R.U. Monserrate, and B.O. Juliano 1973. Protein Metabolism in Leaves and Developing Grain of Rices Differing in Grain Protein Content. Plant Physiol. 51: 537-542.

Pesci, P. 1989. Involvement of CL^- in the Increase in Proline Induced by ABA and Stimulater by Potassium Chloride in Barley Leaf Segments. Plant Physiol. 89: 1226-1230.

Peterson D.M., schrader L.E., Cataldo D.A., Yonus V.L. and Smith D. 1975. Assimilation and Remobilization of Nitrogen and Carbohydrates in Oats, Especially As Related to Grain Protein Concentration. Can. J. Plant Sci. 55: 19-28.

Poovaiah B.W. and Veluthambi K. 1985. Auxin-regulated Invertase Activity in Strawberry Fruits. Journal of the American Society of Horticultural Science. 110: 258-261.

Portch S., MacKenzie A. F. and Steppler H. A. 1968. Effect of Fertilizers, Soil Drainage Class and Year upon Protein Yield and Content of Oats. Agron. J. 60: 672-675.

Rademacher W. and Graebe J.E. 1984. Hormonal Changes in Developing Kernels of two Spring Wheat Varieties Differing in Storage Capacity. Berichte Deutschen Botanischen Gesellschaft 97: 167-181.

Rao, S.C. and L.I. Croy 1972. Protease and Nitrate Reductase Seasonal Patterns and Their Relation to Grain Protein Production of 'High' vs 'Low' Protein Wheat Varieties. J. Agr. Food Chem. 20:

Rao, S.C. and L.I. Croy 1971. Protease Levels in 'High' Versus 'Low' Grain Protein Wheats and Their Association with the Production of Amino Acids, Tryptophan, and IAA During Early Growth. Crop Sci. 11: 790-791.

Rasmusson Donald C. 1985. Barley. pp. 187-230. American Society of Agronomy, Inc.

Reed A.J. and Singletary G.W. 1989. Roles of Carbohydrate Supply and Phytohormones in maize kernel abortion. Plant-Physio. Rockville, Md. : American Society of Plant Physiologists. 91: 986-992.

Salsac L., Sylvain C., Morot-Gaudry J.F., Lesaint C. and Jolivet E. 1987. Nitrate and Ammonium Nutrition in Plants. Plant Physiol. Biochem. 25 (6): 805-812.

SAS Institute. 1985. SAS User's Guide. Statistics. Version 5 ed. SAS Inst., Cary, NC.

Schussler J.R., Brenner M.L. and Brun W.A. 1984. Abscisic Acid and its Relationship to Seed Filling in Soybeans. Plant Physiology. 76: 301-306.

Seemann J. R. and Sharkey T. D. 1987. The Effect of Abscisic Acid and other Inhibitors on Photosynthetic Capacity and the Biochemistry of CO_2 Assimilation. Plant Physiol. 84: 696-700.

Scott R.K. and Dennis-Jones R. 1976. The Physiological Background of Barley. Journal of the National Institute of Agricultural Botany. 14: 182-187.

Shewry P.R., Field J.M., Kirkman M.A., Faulks A.J. and Miflin B.J. 1980. The Extraction, Solubility, and Characterization of Two Groups of Barley Storage Polypeptides. J. Exp. Bot. 31: 393-407.

Shewry P.R. 1992. Barley : Genetics, Biochemistry, Molecular Biology and Biotechnology. pp. 403-411. C.A.B. International.

Simpson R.J. and Dalling M.J. 1981. Nitrogen Redistribution During Grain Growth in Wheat (Triticum aestivum L.) III. Enzymology and Transport of Amino Acids From Senescing Flag Leaves. Planta 151: 447-456.

Simpson R.J., Lambers H. and Dalling M.J. 1983. Nitrogen Redistribution During Grain Growth in Wheat (Triticum aestivum L.) IV. Development of a quantitative model of the Translocation of Nitrogen to the Grain. Plant Physiol. 71: 7-14.

Smith T.L., Peterson G.A. and Sander D.H. 1983. Nitrogen Distribution in Roots and Tops of Winter Wheat. Agron. J. 75: 1031-1036.

Soares M. I. M. and Lewis O. A. M. 1986. An Investigation into Nitrogen Assimilation and Distribution in Fruiting Plants of Barley (Hordeum Vulgare L. cv. Clipper) in Response to Nitrate, Ammonium and Mixed Nitrate and Ammonium Nutrition. New Phytol. 104: 385-393.

Spiertz J.H.J. and Ellen J. 1978. Effects of Nitrogen on Crop Development and Grain Growth of Winter Wheat in Relation to Assimilation and Utilization of Assimilates and Nutrients. Neth J Agric Sci. 26: 210-231.

Stance, A.M., V. Terzi and L. Cattivelli 1992. Biochemical and Molecular Studies of Stress Tolerance in Barley. In: Barley: Genetic, Biochemistry, Molecular Biology and Biotechnology. (Ed. P.R. Shewry). pp. 277-283. C.A.B. International.

Steel R. G. and Torrie J.H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York.

Steward A. Brown and Neish A. C. 1953. The Biosynthesis of Cell Wall Carbohydrates Glucose-C^{14} as a Cellulose Precursor in Wheat Plants. Can. J. of Biochem. and Physiol. 32:

Stoskopf C. Neal. 1985. Creal Grain Crops. pp. 123-124. Reston Publishing Company, INC.

Thorpe T.A., Bagh K., Cutler A.J., Dunstan D.I., McIntyre D.D. and Vogel H.J. 1989. A 14N and 15N Nuclear Magnetic Resonance Study of Nitrogen Metabolism in Shoot-forming Cultures of White Spruce (Picea glauca) Buds. Plant Physiol. Rockville, Md. : American Society of Plant Physiologists. Vol: 91 (1) p. 193-202.

Tomkins J.P. and Hall M.H. 1991. Stimulation of Alfalfa Bud and Shoot Development With Cytokinins. Agron-J. Madison, Wis. : American Society of Agronomy. 83: 577-581.

Tully R.E. and Hanson A.D. 1979. Amino Acids Translocated From Turgid and Water-stressed Barley Leaves. I. Phloem Exudation Studies. Plant Physiol. 64: 460-466.

Walton D.C. 1980. Biochemistry and Physiology of Abscisic Acid. Annual Review of Plant Physiology. 31: 453-489.

Warder E.G., Lehane J.J., Hinman W.C. and Staple W.J. 1963. The Effect of Fertilizer on Growth, Nutrient Uptake and Moisture Use of Wheat on Two Soils in South-western Saskatchewan. Can. J. Soil Sci. 43:107-116.

Wareing P.F. and Seth A.K. 1967. Ageing and Senescence in the Whole Plant. Soc. Exp. Biol. Symp. 21:543-558.

Waters S.P., Noble E.R. and Dalling M.J. 1982. Intracellular Localization of Peptide Hydrolases in Wheat (Triticum aestivum L.) Leaves. Plant Physiol. 69: 575-579.

Zadoks J.C., Chang T.T. and Konzak C.F. 1974. A Decimal Code for the Growth Stages of Cereals. Weed Res. 14: 415-421.

Zhang F., Mackenzie A.F. and Smith D.L. 1993. Corn Yield and Shifts Among Corn Quality Constituents Following Application of Different Nitrogen Fertilizer Sources at Several Times During Corn Development. J. of Plant Nutrition. 16(7); 1317-13337.

Zieserl J.F. JR. and Hageman R.H. 1962. Effects of Genetic Composition on Nitrate Reductase Activity in Maize. Crop Sci. 2: 512-515.

Zieserl, J.F., W.L. Rivenbark, and R.H. Hageman 1963. Nitrate Reductase Activity, Protein Content, and Yield of Four Maize Hybrids at Varying Plant Populations. Crop Sci. 3: 27-32.